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Construction of a Chemical-Microbial Pilot Plant for Production of Single-Cell Protein from Cellulosic Wastes

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The design, construction, and operation of a pilot-plant unit to convert waste sugarcane bagasse into microbial single-cell protein by a previously developed process is described. After the bagasse was ground and given a milk alkaline oxidation treatment it was fermented on a continuous-flow basis by Cellulomonas, sp bacteria, and also by a mixed culture run of Cellulomonas and the symbiotic organism *Alealigenea faecalis*. Maximum cell density obtained with pure Cellulomonas was 1.7 g dry weight per liter and 6.24 g per liter for the mixed culture. Culture mass-doubling times during log-phase growth were usually from 3.2 to 3.7 hr. The maximum experimental volumetric production efficiency of a continuous run using pure Cellulomonas was about 0.10 g of dry cell mass per liter of fermenter capacity per hr. The single-cell protein, produced as a light brown to yellow-brown powder, has a crude protein content of 50 to 55 percent.

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CONSTRUCTION OF A CHEMICAL-MICROBIAL PILOT PLANT FOR PRODUCTION OF SINGLE-CELL PROTEIN FROM CELLULOSIC WASTES

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1971

FOREWORD

The 1965 Solid Waste Disposal Act (Public Law 89-272) as amended by the Resource Recovery Act of 1970 (Public Law 91-512), placed great emphasis on the recovery and reuse of resources now being wasted. Cellulose represents more than 50 percent of municipal refuse and a much larger fraction of agricultural wastes. As the use of paper and paper products grows, the amount of cellulose waste also increases.

Louisiana State University, under contract to the Federal solid waste management program of the U.S. Environmental Protection Agency, has constructed and operated a pilot plant for the fermentative production of bacterial single-cell protein from cellulosic wastes. This plant was constructed at the Mississippi Test Facility (MTF) of the National Aeronautics and Space Administration (NASA), whose participation and aid greatly enhanced the value of the project. This report on the results of that contract (No. PH 86-68-152) was prepared by the Department of Chemical Engineering of the University. The Federal solid waste management program was represented by Thomas C. Purcell and Clarence A. Clemons during the implementation of the contract and the preparation of the report.

--RICHARD D. VAUGHAN
Deputy Assistant Administrator
for Solid Waste Management

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SUMMARY

A pilot plant was designed, constructed, and operated that produced microbial single-cell protein from waste sugarcane bagasse. Bagasse was ground and given a mild alkaline-oxidation treatment before fermentation Bagasse was slurried at as much as 10 g per liter dry weight in water with a simple nutrient salts mixture to form the fermenter feed stream. The process was operated in both batch and continuous-flow patterns.

Cellulomonas, sp. bacteria were used in a pure culture for most runs, but a mixed culture run of Cellulomonas and the symbiotic organism *Alcaligenes faecalis* showed much higher production capabilities. Maximum cell density obtained with pure Cellulomonas was 1.7 g dry weight per liter and 6.24 g per liter for the mixed culture. Culture mass-doubling times during log-phase growth were usually from 3.2 to 3.7 hr.

The maximum experimental volumetric production efficiency (VPE) of a continuous run using pure Cellulomonas was about 0.10 g of dry cell mass per liter of fermenter capacity per hr. The mixed culture run had a theoretical VPE of 0.512.

Single-cell protein was produced as a light brown to yellow-brown powder and had a crude protein content of 50 to 55 percent.

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions were drawn from data and experience obtained during the fulfillment of contract PH#86-68-152.

1. Alkali-treated sugarcane bagasse can be fermented on a continuous-flow basis by Cellulomonas, sp. bacteria for the production of single-cell protein.
2. Cellulomonas will preferentially metabolize soluble carbohydrate rather than insoluble cellulose if both are present in the media.
3. Bagasse must be subjected to alkali treatment before appreciable bacterial attack can occur.
4. As much as 90 percent of treated bagasse can be solubilized in batch fermentations, but not all of this is metabolizable carbohydrate.
5. About 26 percent of whole, bone-dry bagasse is converted to cell mass at a continuous fermenter efficiency of 75 percent.
6. Usual log-phase culture mass-doubling time for Cellulomonas is 3.2 to 3.7 hr.
7. Increased agitation increases final cell density and growth rate probably by improving oxygen and substrate mass transfer.
8. Fertilizer-grade and industrial-grade chemicals may be used in most cases to replace laboratory-grade or reagent-grade salts used in the nutrient media.
9. The presence of alkali in the feed stream improves the efficiency of continuous sterilization.

10. Mixed-culture fermentation with Cellulomonas and *Alcaligenes faecalis* gives much higher cell density than a pure Cellulomonas culture.
11. The maximum cell density obtained with a pure Cellulomonas culture is 1.66 g per liter dry weight. The mixed-culture run had a cell density of 6.24 g per liter.
12. Maximum experimental volumetric production efficiency obtained by a pure Cellulomonas culture in a continuous run was 0.098 g of dry cell mass per liter of fermenter volume per hour. Calculated VPE for the mixed culture was 0.512 g per liter per hour.
13. Cellulomonas contains about 50 to 55 percent crude protein (Kjeldahl method) and has a good amino acid balance.
14. Passage of the alkali-treated solids through the oven was found not to be necessary to assure degradation by the organisms.

The following recommendations have been made in light of experience gained during the performance of the contract.

1. Further work needs to be done in defining the limits and capabilities of mixed-culture fermentation of cellulose.
2. The pilot plant should be modified to permit less severe alkali treatment of the cellulosic before fermentation.
3. A second fermentation vessel should be added to the pilot plant to permit two-stage fermentation.
4. A harvesting method needs to be perfected that will give a clean cellulose-free cell product that is low in salt.
5. Further work needs to be done on recycle and use of the solid and liquid by-product streams.

6. An automatic antifoam addition system needs to be added to the fermenter, and concurrent analysis of cell density and substrate concentration should be perfected.
7. The infrared oven should be removed as a pre-fermentation processing step in the treatment of bagasse.

CONSTRUCTION OF A CHEMICAL-MICROBIAL PILOT PLANT FOR THE PRODUCTION OF SINGLE-CELL PROTEIN FROM CELLULOSIC WASTES

The 1970's have been introduced as a decade of conservation and environmental reclamation. It is fervently hoped that this will prove to be the case. The life-supporting resources of the earth and its atmosphere are being depleted and polluted at an alarming rate while the population increases ever more quickly. The use of the earth's ecosystem by man has resulted in the absolutely predictable problem of too much waste and too few resources. The historical role of industry has been one of processing raw materials into usable consumer goods. The inevitable fate of these goods has been to become wastes. Nature is then left with the task of reconverting these wastes into resources.

Increasing population and industrialization have increased waste output to a point where natural reclamation pathways cannot keep up. The synthesis pathways of nature are overloaded. It is clear that new methods of waste reclamation and reuse must be developed. The usable lives of raw materials must be lengthened, and more efficient ways of returning wastes to resources must be found.

In September 1968, the Department of Chemical Engineering of Louisiana State University, under contract to the Bureau of Solid Waste Management of the Department of Health, Education, and Welfare, began the design and construction of a pilot-plant unit for the conversion of waste cellulose materials into bacterical single-cell protein (SCP) by fermentation.

Prior laboratory work had shown that the proposed process was technically feasible, and the preliminary economic data were encouraging. The scope of the contract was to design, construct, and operate a pilot unit; produce bacterial single-cell protein for analysis and testing; and evaluate process economics more completely. The initial operation of the pilot unit has been limited to a single waste cellulosic substrate, sugarcane bagasse. This material represented a rather typical heterogeneous cellulosic agricultural waste, was easily accessible, and was a good example of an under-utilized raw material. Purified ground wood pulp was also used as a control substrate in several runs.

The process was designed for a dual purpose. The first was the use of present wastes and potential pollutants; the second was the production of inexpensive, high-quality protein for food. The success of the idea may be judged from the tremendous interest and response this project has received from industry; the success of the process will be proved by its future industrial exploitation.

AVAILABILITY OF CELLULOSIC WASTES

Cellulose is by far the most widespread and readily available of all solid organic materials. It comprises almost one-third of the weight of all trees, vines, grasses, and straws. Unlike other resources such as oil and minerals, cellulose is constantly replenishing itself by photosynthesis and growth. Vast quantities of this material accumulate as waste products from activities such as food processing, lumbering, paper making, and cereal grain harvesting. Additionally, municipal and industrial wastes of paper, rags, boxes, wood, excelsior, grass, and leaves raise the available amount of cellulose-bearing material to astronomical proportions.

The ready availability of such a quantity of cellulose has been the impetus for voluminous research into novel and diverse methods of utilizing this material. Wallboard, door cores, and mulch have been made from cereal grain straws and sugarcane bagasse; chemical-grade cellulose from cotton linters, wood, and bast fibers; animal feed pellets from pea vines and other fibrous vegetable roughages; animal bedding from oat, rice, and wheat straw; furfural and brake shoes from sugarcane bagasse; various chemicals from corn cobs; and limitless other examples. Still, most waste cellulose is dumped, buried, burned, or used as a fuel supplement; and its chemical energy or physical utility is thereby wasted or only fractionally retained.

Cellulose is a major constituent of all woody plants, grasses, and vegetables. The few plants that have become industrially important for their cellulose--trees, cotton, flax, etc--have been chosen, in most cases, for their physical rather than chemical natures.[1]

The availability of waste cellulose may be brought into better perspective by considering urban or municipal wastes and agricultural wastes separately. Cellulose found in urban wastes; for example, paper, rags, cardboard, has usually been subjected to some type of processing, while cellulose agricultural wastes are almost always found in the native, heterogeneous state.

Urban Wastes

The United States alone produces more than 250 million tons of urban wastes per year.[2] Cellulose comprises from 40 to 50 percent of this total.[3] Most of the cellulose of urban waste is from paper, while leaves, grass, and wood supply most of the rest.[4] Only 18 to 20 percent of the waste paper is reclaimed and reused for paper stock, and all of the rest is either incinerated or used as landfill.[5]

Of the cellulose found in urban refuse, much more than 20 percent could be reused in paper making. Japan currently reuses more than 40 percent of her urban waste cellulose.[5] Much of the cellulose in urban wastes is, however, either of such poor fiber quality or so intimately mixed with noncellulosics that it is not suitable for recovery and reuse. Yet, if this cellulose could be used for its chemical rather than physical properties, a far greater portion could be removed from incinerators and landfills.

The yearly appearance of about 70 million tons of cellulose in urban wastes, less about 10 million tons reclaimed for reuse, leaves 60 million tons per year net waste cellulose in the United States alone.

Agricultural Wastes

The U. S. Department of Agriculture has estimated that more than 200 million tons of cellulosic agricultural wastes such as plant stems, straw, leaves, grasses, bagasse, and husks are produced every year in the United States.[4] More wastes come from canning and food packaging and preparation plants. Less than 1 percent of these agricultural wastes are used; most are left in the fields to rot. Some of these wastes accumulate at some central point during processing. Sugarcane bagasse, sugar beet vinasse, rice and wheat husks, corn cobs and husks, and several others are generally brought to a central point in their usual processing cycle. When these materials accumulate, they present a disposal problem and rank as pollutants simply by their volume and lack of profitable use. Much of this material is burned as fuel to fire plant boilers. Some, however, is reused and made into various construction materials and agricultural products.

Total world production of sugarcane bagasse is about 36 million tons yearly.[6] The United States contributes about 13 million tons. Most of this is burned as boiler fuel in the sugar mill, but some is used for paper stock, hardboard, furfural production, and charcoal.

Economics of Cellulose Wastes

Since waste materials do not usually enter the economic or industrial cycle, it is often difficult to quote a price or value for them. Because they are of rather low economic value, the costs of handling, storage, transportation, and preparation become large factors. Some cellulosic waste like mixed urban refuse can be attributed a negative cost, usually equal to the disposal costs, and the value of some is determined as fuel replacement value.

The cost of sugarcane bagasse in the United States ranges from about $6 to $13 per ton baled.[7] The bagasse contains about 50 percent moisture. Cellulose makes up from 50 to 60 percent of bone-dry bagasse, and hemicelluloses add from 10 to 20 percent. The remainder is largely lignin and ash. The mill cost of bone-dry bagasse carbohydrate then is from $15 to $40; an average cost would be about $20 per ton of bone-dry carbohydrate from bagasse. Added to this cost are the costs of baling, handling, storage, and short-haul transportation. This usually adds about $10 per ton. Total cost would therefore be about $30 per ton, or 1.5 cents per lb of fermentable carbohydrate from bagasse. The cost of Number One Mixed Grade waste paper is about the same.[4]

In every case dealing with waste cellulose reuse, the cost of transportation makes it necessary to limit hauling distances to a minimum. If costs are calculated on materials such as cereal grain straws, then gathering costs must also be added. Yet, with a maximum cost of 1.0 to 1.5 cents per lb, low-grade waste cellulose remains a relatively inexpensive raw material.

THE PROBLEM OF PROTEIN

Coupled with the problem of increasing waste loads is the problem of dwindling resources. A major resource in short supply now appears to be food, and more particularly, protein.

The scope of the world food problem up to the present has been limited mainly to the underdeveloped continents of Asia, Africa, and Latin and South America. These nations face a staggering food deficit before 1975. Predictions show, however, that the more developed nations will eventually face the same problems. Food and Agricultural Organization (FAO) estimates for nations such as Pakistan and India run well over 100 percent beyond current demands.[8]

In particular, the problem of protein deficiency has received much interest since high-quality protein is in such shortage in countries like India, Pakistan, and Brazil.[9,10] Protein demand for the future largely follows the predictions for calory requirements. The protein problem is further complicated by the protein quality factor. To be of usable metabolic quality, protein must contain all the amino acids necessary for growth, maintenance, and reproduction in a balance suitable for efficient use. This high-quality protein has been supplied almost entirely by meat, milk, fish, eggs, and poultry. Present trends show, however, that population growth and increasing food demands will severely exceed protein supply from these sources. Better animal husbandry is certainly possible, especially in the underdeveloped countries, but that alone cannot cope with increasing requirements. Technology is faced then with the problem not only of increasing the world protein supply by 50 percent in the next 20 years, but also of producing protein of low cost, high quality, easy distribution, and high social acceptability.

The process developed at Louisiana State University (LSU) is the fermentation of insoluble cellulose by a cellulolytic bacterium. The bacteria are then harvested from the media for use as a food protein. The process was developed for the utilization of excess sugarcane bagasse, and bagasse has been retained as the sole carbon source for most of the pilot unit runs. The SCP produced is a light brown-to-yellow powder having a crude protein content of from 50 to 60 percent. The SCP has a good amino acid pattern and has served as a protein source in successful rat-feeding studies. More of the economic aspects, quality considerations, and market properties of this SCP product will be discussed later in this report.

The term fermentation, as used in this report, means the respiration and subsequent growth of a bacterial culture under aerobic conditions.

INITIAL DEVELOPMENT OF THE PROCESS

Studies on the sources of protein show that protein production rates and efficiencies differ widely. Differences in protein production rates were demonstrated by Thaysen with the example that a 1,000-lb bullock can synthesize 0.9 lb of protein every 24 hr, whereas 1,000 lb of soybeans synthesize 82 lb of protein in the same length of time, and 1,000 lb of yeast could produce more than 50 tons of protein in 24 hours.[11] The difference in the efficiency of protein production between, for example, the bullock and yeast growth is equally marked. If carbohydrate were fed to both, the amount ultimately formed into protein would be no greater than 5 percent in the bullock but more than 25 percent in the yeast.

Such drastic difference in production rates and efficiencies have claimed the interest of many investigators whose proposals have, in some way, been concerned with either shortening the protein production chain or increasing the efficiency of one or more of the steps.

A comparison of the land area necessary for the support of each of these protein-producing activities would show again the savings and efficiency enjoyed by the yeasts. In the LSU process, a bacterium was used, and the carbohydrate source was cellulose.

Cellulose Properties and Treatment

The natural carbohydrates that occur in plants are of a rather motley character. Many different sugar molecules may be found; and these, in the native cellulosic, are bound together by several different types of linkages. The classical definition of cellulose is a linear polymer of anhydroglucose units linked at the one and four carbon atoms by a beta-glucoside bond. The number of repeating units may range from about 30 to 5,000 or more. The highest degree of polymerization (DP) recorded is about 15,000 units. In native celluloses the polymer chains are of widely varying lengths; and so, polymer weights and degrees of polymerization differ throughout a sample.

In addition to the carbohydrates, these native agricultural wastes also contain lignin, protein, fat, and ash. A proximate analysis gave the relative fractions of these constituents in several different types of cellulosic agricultural wastes (Table 1).

The macromolecular physical structure, or fine structure, of a cellulosic material in the native solid state is a complex function of intermolecular and intramolecular forces between and within individual cellulose polymer chains; between cellulose fibrils in fibers; and between fiber units and the hemicelluloses, lignin, gums, resins, and minerals. Since formation of the physical structure of cellulose occurs as a growth process, the interrelationship of the various components is in a dynamic and ever-changing state.

H. Mark states that all properties of cellulose (both chemical and physical) are, in the last analysis, determined by chemical structure but that forces between the cellulose polymer chains produce a super-molecular texture that profoundly influences most properties of the material.[12]

General agreement has been reached on the more important points of gross physical structure of cellulose as follows:

1. The polymer chains of natural cellulose exist in differing degrees of order with respect to each other.

TABLE 1

PROXIMATE ANALYSIS OF AGRICULTURAL CELLULOSICS[1]

Substrate	Protein	Fat	NFE*	Fiber	Lignin	Ash
Cotton linters - raw	2.45	0.96	4.48	85.7	5.0	1.27
Cotton linters - purified	3.27	1.69	3.69	90.0	0.84	0.42
Bagasse - whole	2.68	1.0	31.1	52.74	10.63	1.79
Bagasse - pith	2.97	5.23	30.83	47.14	9.28	4.52
Bagasse - fiber	1.74	0.76	32.78	52.39	10.23	2.06
Rice straw	5.05	2.41	36.70	34.39	4.06	17.36
Sawdust - pine	0.42	2.89	13.16	74.83	8.45	0.21
Johnson grass	6.71	2.87	39.44	37.00	5.01	8.95
Prairie grass	5.32	2.06	41.84	36.73	7.39	6.63
Alfalfa meal	22.11	2.38	39.46	23.88	5.39	6.74
Cottonseed hulls	5.53	1.51	15.83	50.54	23.31	3.25
Corn cobs	2.61	0.87	46.89	37.10	10.44	2.06
Oat straw	7.74	2.0	35.50	36.94	7.41	10.39
Wheat straw	2.71	1.25	35.58	46.08	8.02	6.36
Sorghum bagasse	2.73	2.73	41.60	40.54	7.17	4.21

*NFE is Nitrogen-Free Extract.

2. The most highly ordered fraction of cellulose gives a definite and unique crystalline diffraction pattern in x-ray diffraction photographs. The exact size and lattice angles may be computed for a characteristic single crystal.
3. The least ordered fraction is entirely amorphous and shows no regularity whatsoever.
4. The crystalline fraction, whether composed of discrete crystallites or of a continuous nature, is difficultly penetrable by solvents, enzymes, or reagents.
5. The amorphous region, whether interstitial or sequential, or both, is easily penetrable by solvents, enzymes, or reagents.
6. Cellulose in either region has the same chemical structure.

The relative crystallinites of several cellulosics, determined by the x-ray diffraction technique of Segal[13] are listed here.[1]

Cotton linters, raw	72.8
Cotton linters, purified	81.0
Bagasse - whole	42.5
Bagasse - pith	32.3
Bagasse - fiber	42.3
Rice straw	43.3
Sawdust - pine	38.0
Johnson grass	33.3
Prairie grass	39.2
Alfalfa meal	33.3
Cottonseed hulls	42.0
Corn cobs	28.6
Oat straw	38.7
Wheat straw	46.6
Sorghum bagasse	42.2

It is evident that relative crystallinities differ widely among the materials, whole sugarcane bagasse being in an intermediate position. Several investigators have noted that the reactivity or enzyme degradation of cellulose varies inversely with its crystallinity.[14]

An important property of cellulose is that it can be penetrated and swollen by some strongly electrolytic solvents. This phenomenon has long been used in treatment of cellulose to increase reactivity, to change the solid physical state, and to improve dyeability and surface properties. A partial list of chemical agents that will penetrate and swell cellulose to some degrees follows.

9% sodium hydroxide + carbon disulfide
Calcium thiocyanate
Cuprammonium hydroxide
Sodium hydroxide
Sulfuric acid
15% sodium hydroxide + carbon disulfide
Ruthenium red
Copper ethylendeiamine
Phosphoric acid
85% formic acid + zinc chloride (80:20) AmZn
Trimethylbenzylammonium hydroxide
Iron tartrate complex
Methacrylate embedding
Sodium zincate
Cadoxen

Of the reagents listed, cuprammonium hydroxide, sodium hydroxide, sulfuric acid, and copper ethylenediamine have received most interest and industrial application. Other chemical agents such as nitrogen dioxide in dimethylsulfoxide, and zinc chloride have recently gained industrial interest. An excellent review of the literature on action of cellulose-swelling agents has been published.[15]

The importance of the swelling action of some reagents upon cellulose has been realized for a long while. Almost all processes that involve reactions of cellulose utilize either a pretreatment to swell the cellulose or a reaction solvent that also acts as a swelling agent. The formation of alkali-cellulose is a predominant intermediate step in the courses of many cellulose substitution reactions, and the catalytic nature of alkali in cellulose degradation is well respected.

The degradation of cellulose is generally understood to mean a decrease in the average degree of polymerization of the polymer. McBurney has characterized the four major types of cellulolytic degradation:[16]

1. Hydrolytic - cellulose is reduced in DP and shows an increase in reducing power.
2. Oxidative - cellulose is reduced in DP, and the product shows a development of carbonyl and carboxyl groups.
3. Microbiological - cellulose may be reduced in DP, but loss of strength is the most pronounced effect.
4. Mechanical - cellulose is reduced in DP if the fiber is subjected to severe physical treatment.

Cellulose can be degraded microbially by microorganisms or enzyme systems of these organisms. Ruminant animals, termites, some snails, and many bacteria and fungi depend upon their ability to degrade cellulose and metabolize its degradation products for their supply of carbon and energy. In each case the instrument of degradative action is an enzyme or enzyme system produced by the organism.

The ideas used in the design and development of the pre-fermentative treatment of the cellulosic wastes for the LSU process were directed towards making the cellulose more bio-degradable. The criteria of the treatment process were:

1. To decrease the relative crystallinity of cellulose
2. To disrupt the physical structure of the lignin in the material
3. To decrease the average DP of the cellulose
4. To obtain these changes at a competitive cost.

A mild alkaline swelling of the cellulose followed by an air oxidation seemed to meet all these criteria. Sodium hydroxide is available at low cost from by-product streams of chlorine manufacture. It swells the cellulose and solubilizes part of the lignin. The swelling causes the lignin sheathing to be disrupted, and the cellulose is made more accessible to enzymes. If this treatment is followed by an air oxidation, the cellulose is degraded to a lower DP and the relative crystallinity is reduced.

An average component analysis of bagasse before and after the treatment process showed that the carbohydrate fraction remaining in the bagasse after a water extraction was increased from about 57 percent to almost 75 percent. The water-soluble fraction of carbohydrate rose from about 2 percent to almost 18 percent. This rise in relative carbohydrate content was caused by preferential removal of noncarbohydrate components such as resins, gums, lignin, fat, protein, and dirt. The increase in water-soluble carbohydrate was caused by the oxidative de-polymerization of a fraction of the cellulose present.

Bagasse showed that a threefold decrease in DP during the treatment, averaging a DP of more than 800 before treatment and less than 300 after. The overall degree of relative cellulose crystallinity also was lowered from about 50 percent to 10 percent by the treatment.

Ten different cellulosics were treated by alkaline-oxidation, and the biodegradability of their cellulose fractions was determined by the *in vitro* rumen fluid method of Baumgardt.[17] The treatment increased cellulose digestibilities on an average of 85 percent (Table 2).

Cellulose Fermentation

All industrial use of carbohydrates for microbial substrates has been limited to those that are water soluble. There has been considerable

TABLE 2

IN VITRO RUMEN FLUID DIGESTIBILITIES OF CELLULOSIC WASTES BEFORE AND AFTER TREATMENT[18]

	(% Dry cellulose digested)	
	Untreated	Treated
Bagasse - whole	15.1	57.0
Bagasse - pith	26.5	55.0
Bagasse - fiber	30.1	50.3
Rice straw	7.3	54.1
Johnson grass	66.5	88.0
Prairie grass	16.2	45.7
Corn cobs	19.3	44.0
Oat straw	35.5	66.0
Wheat straw	25.4	44.0
Sorghum bagasse	30.0	61.5

interest, however, in the utilization of soluble sugar fractions produced from insoluble carbohydrate materials. Since Delbrück in Germany found that food-grade yeast could be produced from solubilized wood sugars, sporadic interest has recurred such as in Germany during World War II.[19,20] The German yeast process produced *Candida utilis* and several strains of lesser importance from wood sugars generated by the sulfuric acid hydrolysis of softwoods. Other yeast plants there and current U. S. plants use waste sulfite liquor from paper pulp processing as a substrate.[21] Processes have also been developed for production of bacterial single-cell protein from spent sulfite liquor.[22]

These processes have, in a sense, used insoluble material as substrate, but in all cases the material has been chemically solubilized before introduction into the actual fermentation process.

Viewed as a fermentable carbohydrate, cellulose differs rather radically from carbohydrate substrates in general use. It is insoluble, it is polymerized by a one-to-four beta-glucosidic linkage, it generally has a highly-ordered crystalline fraction, and it is invariably found closely associated with hemicelluloses and lignin. The use of cellulose as a microbial substrate actually adds but one step to the overall mechanism of carbohydrate fermentation. The cellulose must be solubilized before entering cell metabolic pathways. This solubilization is an enzymatic step catalyzed by the cellulase enzyme system of some bacteria and fungi.

For a long while researchers thought native cellulose was either unfermentable or, at least, very resistant to biodegradation. Langwell,[23] Olson, Petterson, and Sherrard,[24] Acharya,[25] and Fontaine[26] reported native cellulose unfermentable or inhibited to a prohibitive extent by lignin. Virtanin and others found that cellulose was biodegradable but that periods of 3 to 4 weeks were necessary for cellulose breakdown.[27] Hajny, Gardner, and Ritter have reported cellulose biodegradation by thermophiles, but residence times were 2 to 6 days.[28] Grey reported conversion of cellulose to cell tissue in fungi, but again rate is measured in days, and maximum carbohydrate-to-protein conversion efficiencies are 17 percent.[29]

Recently the isolation of several mesophilic and thermophilic bacteria with faster growth rates and of fungi with highly active cellulases has increased the prospects of profitable cellulose fermentation.[30,31,32]

The bacterium used in the LSU pilot fermentation process was isolated and identified by Srinivasan and Han.[32] The following is a summary of the methods used in isolation and characterization of the organism.

Isolation medium consisted of 6.0 g/liter sodium chloride, 1.0 g/liter ammonium sulfate, 0.5 g/liter potassium phosphate dibasic, 0.5 g/liter potassium phosphate monobasic, 0.1 g/liter magnesium sulfate, 0.1 g/liter calcium chloride, with 0.1 percent yeast extract and a strip of filter paper. About 1 g of a rotting sugarcane and soil mixture was inoculated into the isolation media. After 3 to 7 days incubation at 30 C on a reciprocal shaker, a portion of filter paper was transferred into fresh media. This process was repeated several times to enrich the aerobic and mesophilic cellulose-utilizing organisms. The filter paper from the enriched culture was removed, macerated in a small amount of sterile water, and streaked onto plates containing each of the following media: nutrient agar, carboxymethyl cellulose agar, and filter paper agar (a plate of nutrient agar covered with filter paper). Representatives of the various colonies that developed on each of these media were transferred to test tubes containing nutrient salts and cellulose. Tubes showing visible degradation of filter paper were selected and alternately transferred into liquid and solid media in order to enrich and isolate the cellulolytic organism. Isolated colonies were further purifed by the terminal dilution method. The purity of the isolated culture was confirmed by the microscopic examination and colony morphology of several agar plate colonies. The isolated culture was subjected to diagnostic tests for identification of the strains. A characterization comparison was made with the isolated cellulolytic organism and two organisms of the genus Cellulomonas (Table 3). The organism was able to grow well between the temperatures of 25 C and 35 C. Below 20 C and above 40 C, there was a marked decrease in the growth rate. The effect of pH on the growth rate and activity of the cellulase enzyme was determined and a pH optimum was observed between 6.0 and 7.0 (Figure 1).

TABLE 3

DESCRIPTIVE CHART OF CELLULOSE-UTILIZING ORGANISM[32]

	C. flavigena	*C. uda*	Isolate
Morphological characteristics			
Form	Rods, curved	Rods	Rods, short
Size	0.4-0.6μ x 0.7-1.8μ	0.5μ x 1.0-1.5μ	0.3-0.5μ x 0.7-1.2μ
Motility	Nonmotile	Nonmotile	Nonmotile
Gram stain	Variable	Negative	Negative
Cultural characteristics			
Agar slant	Smooth, glistening, opaque, yellow	Moderate, flat, grayish white	Smooth, glistening opaque, yellow
Broth	Uniformly turbid	Uniformly turbid	Uniformly turbid
Gelatin stab	Slow liquefaction	Slow liquefaction	Slow liquefaction
Filter paper in peptone broth	Fibers separate on slight agitation	Fibers separate on slight agitation	Fibers separate on slight agitation
Optimum temperature	28-33 C	28-33 C	25-35 C
Agar colonies	---	---	Bluish, transparent, smooth, flat, circular; grow feebly on nutrient agar

TABLE 3 (Continued)

	C. flavigena	*C. uda*	Isolate
Biochemical characteristics			
Starch	Hydrolyzed	Hydrolyzed	Hydrolyzed
Nitrate	Reduce to NO_2	Reduce to NO_2	Reduce to NO_2
MR test	--	--	Negative
VP test	Negative	Negative	Negative
Indole production	--	--	Negative
Glucose	Acid	Acid	Acid
Lactose	Acid	Acid	Acid
Sucrose	Acid	Acid	Acid
Maltose	Acid	Acid	Acid
Dextrin	--	--	Acid
Starch	Acid	Acid	--

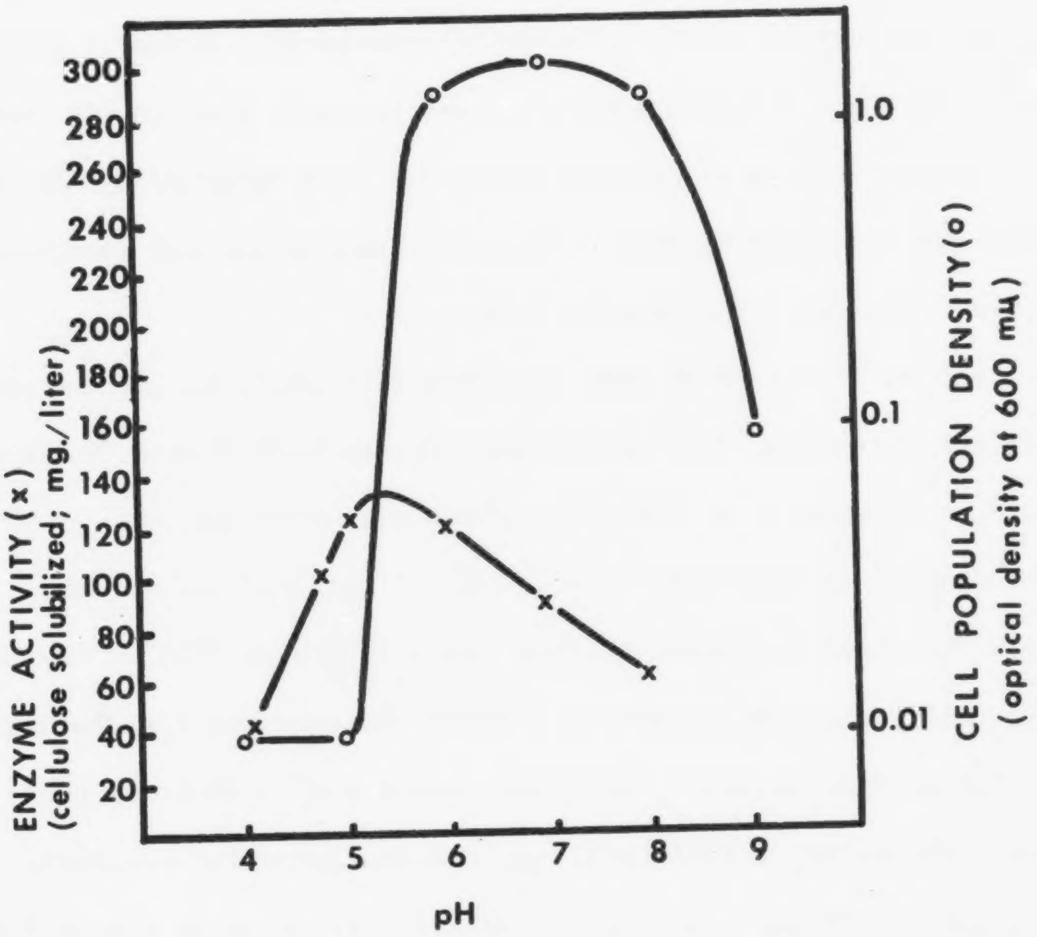


Figure 1. Effect of pH on growth of the organism and activity of the cellulase enzyme.[32]

THE PILOT PLANT

The purpose of the contract was to construct a pilot fermentation unit based on the findings of previous laboratory research. Criteria dictated by both engineering and microbiological practice were used in the design. Many of the larger pieces of process equipment were designed by LSU and constructed and furnished by NASA. Most instrumentation and specialized equipment was obtained with contract funds.

The plant was designed so that fermentations could be carried out in both batch and continuous-flow operation. The general flow sheet (Figure 2) and floor plan (Figure 3) of the pilot plant are about the same as proposed but may be changed to some extent depending on optional use of some equipment. The plant is housed in room 142 of building 8100 at the National Aeronautics and Space Administration's (NASA) Mississippi Test Facility, Bay Saint Louis, Mississippi. This room consists of a 30-ft by 60-ft ceramic tile floor, tile walls, a 16-ft ceiling, a deionized water treatment and storage system (1,500-gal capacity), a 50-gal chilled water system (50 F water at a 90,000 BTU per hour heat load), a more than adequate ventilation system, and a steam generator capable of 600 lb of 100 psi steam per hour. Available utilities consist of 115, 220, and 440 60-cycle AC electrical power; potable water; 90 psi compressed air; and 90 psi compressed nitrogen.

The pilot plant's equipment can be grouped into five distinct process sections: cellulose-handling, treatment, sterilization, fermentation, and cell harvesting.

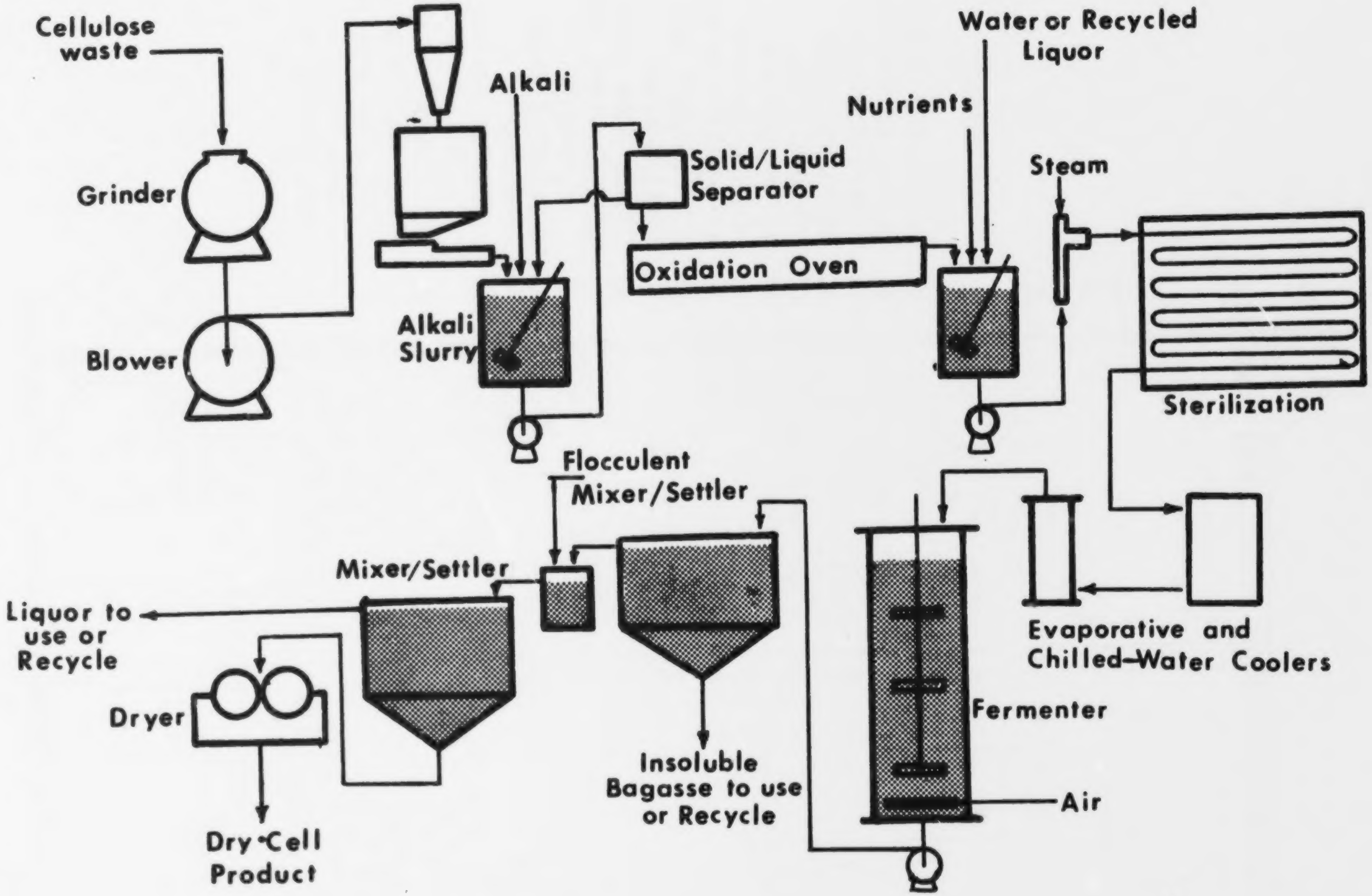
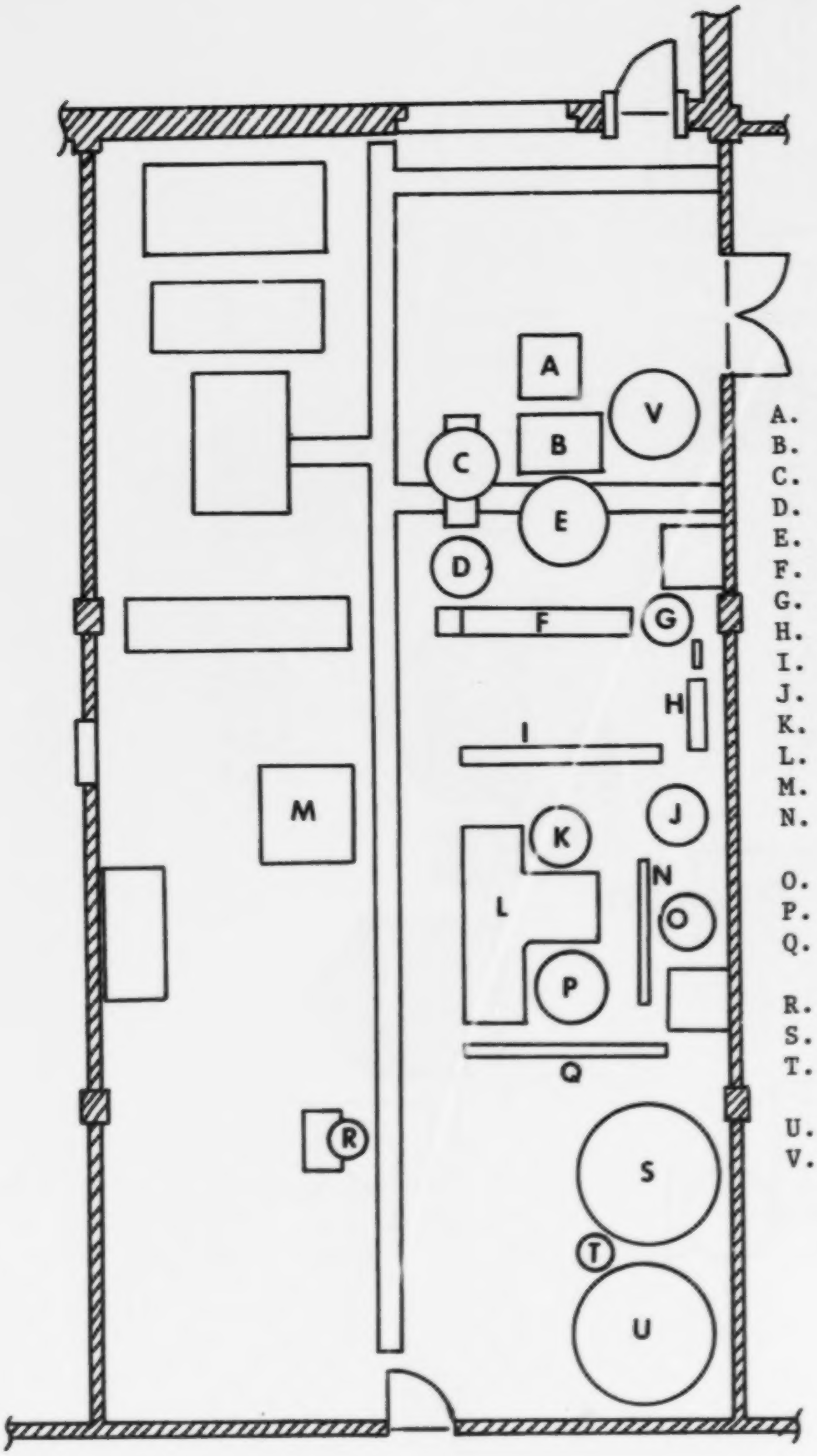

Cellulose waste
Grinder
Blower
Alkali
Alkali Slurry
Solid/Liquid Separator
Oxidation Oven
Nutrients
Water or Recycled Liquor
Steam
Sterilization
Evaporative and Chilled—Water Coolers
Fermenter
Air
Flocculent
Mixer/Settler
Insoluble Bagasse to use or Recycle
Mixer/Settler
Liquor to use or Recycle
Dryer
Dry-Cell Product

Figure 2. Pilot-plant flow sheet.



A. KNIFE GRINDER
B. BLOWER
C. HOPPER AND FEEDER
D. ALKALI SLURRY TANK
E. ALKALI STORAGE
F. OXIDIZING OVEN
G. RESLURRY TANK
H. STEAM INJECTOR
I. HOLDING SECTION
J. EVAPORATIVE COOLER
K. ACID STORAGE TANK
L. WORKING STAND
M. ROTARY DRUM DRYER
N. CHILLED-WATER HEAT EXCHANGER
O. NUTRIENT STORAGE TANK
P. FERMENTER
Q. FERMENTER CONTROL PANEL
R. CENTRIFUGE
S. MIXER/SETTLER NO.1
T. FLOCCULENT ADDITION TANK
U. MIXER/SETTLER NO.2
V. BULK FEED TANK

Figure 3. Pilot-plant floor plan and equipment list.

Cellulose Handling Section

The first step in processing the cellulose is particle size reduction. A five-bladed knife grinder fitted with a 1/8-in. sizing screen is used for this purpose (Figure 4). The five fixed blades and five rotating blades are 18 in. long. The power is supplied by a 7 1/2-hp electric motor that provides for a 300 to 400 lb per hr capacity. The grinder is fitted with a collection hopper and a pneumatic solids-handling pickup attachment. The cellulose is fed in manually and chopped to 1/8-in. particle lengths in this first piece of equipment.

The chopped cellulose is transferred from the grinder to a solids-separating cyclone by the solids blower (Figure 5). The blower is powered by a 7 1/2-hp electric motor that turns a 24-in. fan. The inlet and outlet are approximately 30 sq in., which is more than adequate to handle the maximum grinder through-put.

The solids-separating cyclone collects the ground cellulose and discharges the conveying air out the top port (Figure 6). The cellulose drops into the hopper. The cyclone seems to handle all blower and solids flows and is moderately dust free. No power is consumed in the cyclone operation.

The solids are then collected in a vibratory live-bin hopper (Figure 6). The hopper has a capacity of 15 cu ft, which provides inventory for several hours of cellulose treating. Vibratory action is supplied by a 1-hp electric motor operating an eccentric cam device. The hopper as purchased had no level monitoring system. To remedy this, a vertical slit 1-in. wide was cut in the side of the hopper and a clear plastic plate was used to seal the slit. This provided a convenient, visible means of monitoring hopper level.

NOT REPRODUCIBLE

Figure 4. Knife grinder--initial size reduction.

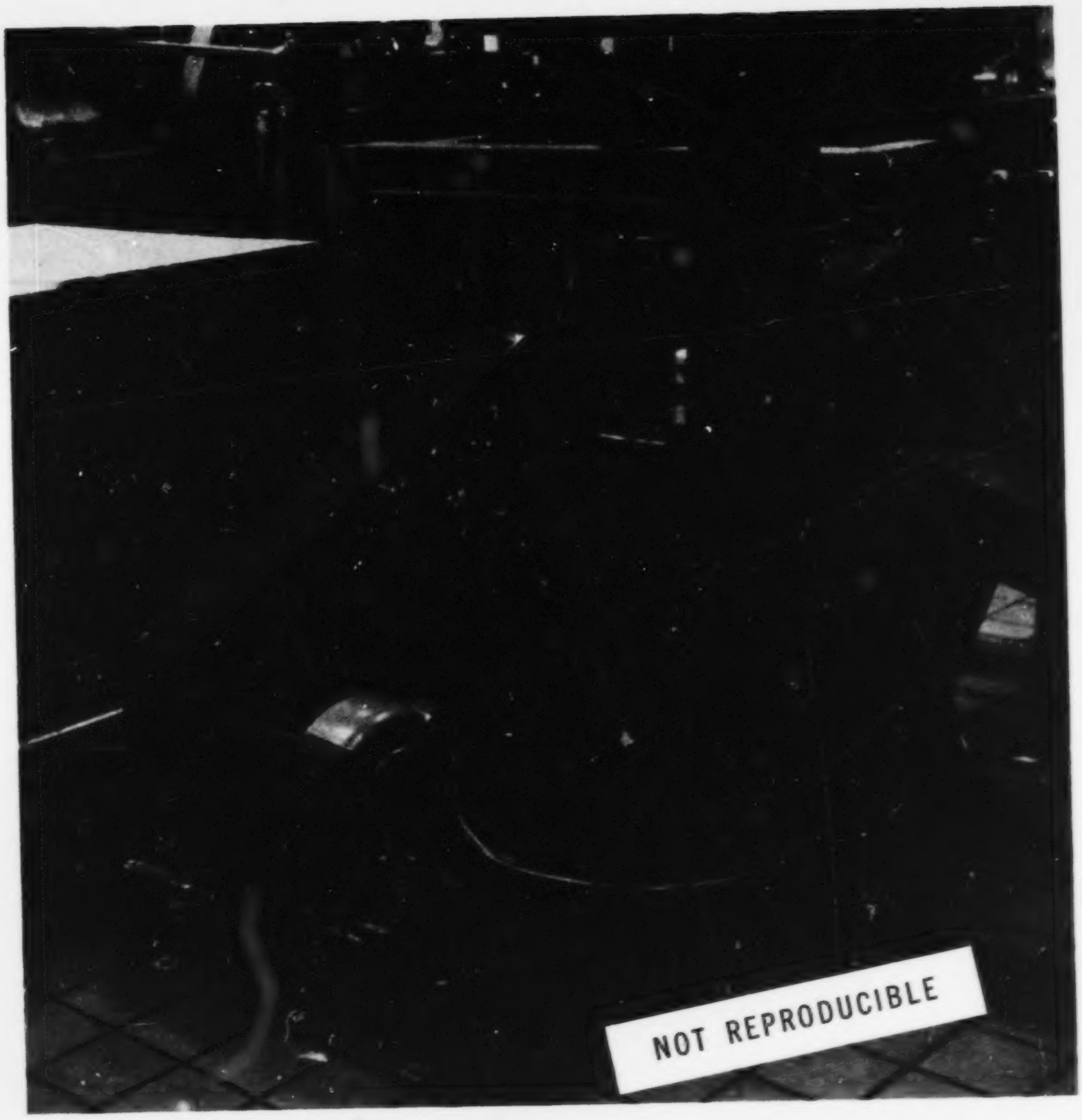

NOT REPRODUCIBLE

Figure 5. Solids blower--air conveying system.

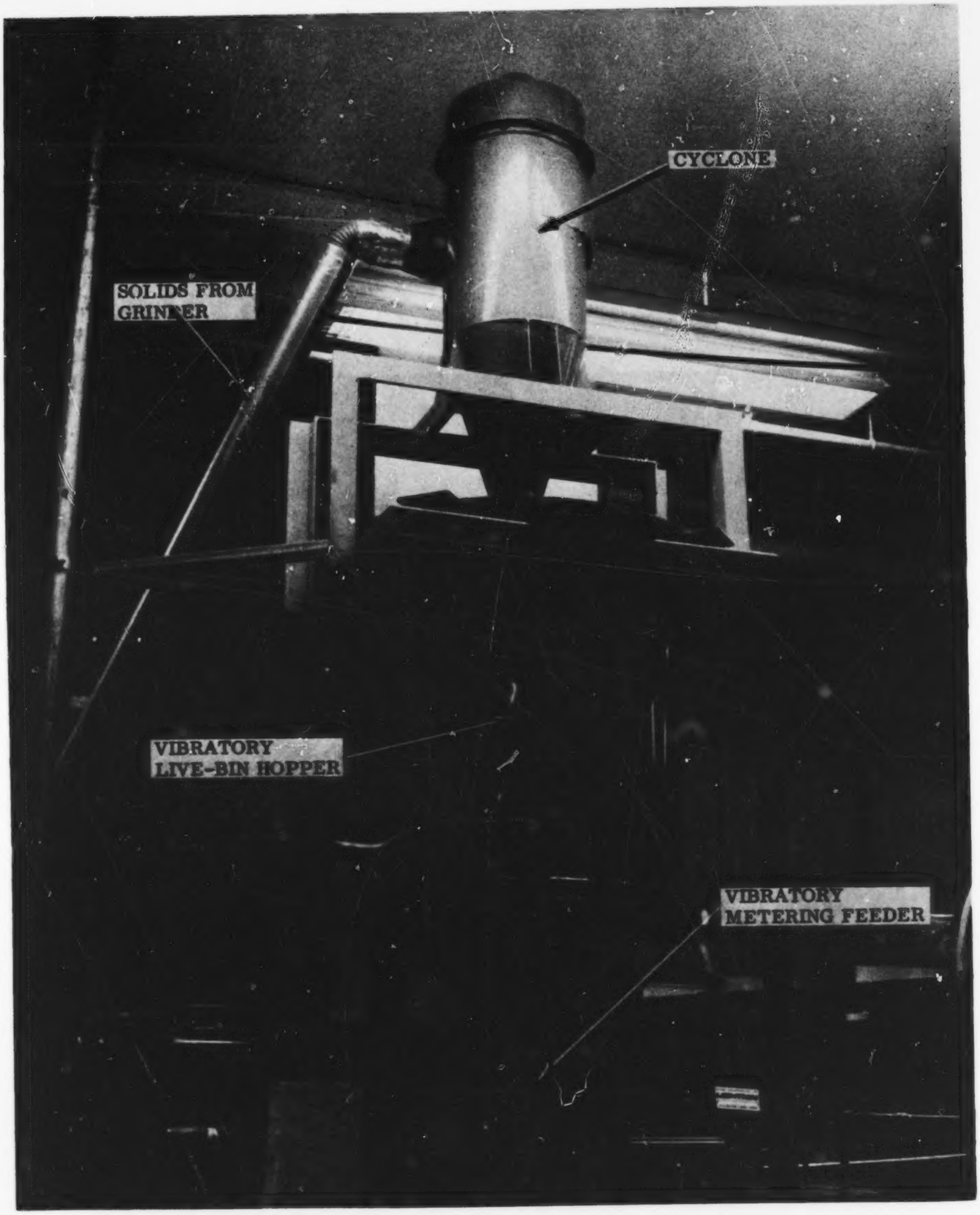

CYCLONE
SOLIDS FROM GRINDER
VIBRATORY LIVE-BIN HOPPER
VIBRATORY METERING FEEDER

Figure 6. Solids cyclone, hopper, and metering feeder.

The vibratory hopper supplies cellulose to the vibratory metering screw feeder (Figure 6). The feeder has a 2-in.-diameter screw powered through a variable-ratio belt and pulley assembly by a 3/4-hp electric motor. The feeding capacity ranges from 0.33 to 2 lb per min of chopped bagasse of bulk density of 5.5 to 6.0 lb per cu ft. This feeder was intended to meter the amount of cellulose being fed into the feed makeup for the feed stream to the fermenter. The required feed rate was found to be much smaller than the range capability of the feeder. The feeder was, therefore, operated on a manual time-cycling basis.

With the exception of the oversized feeder, all equipment in the cellulose handling section worked with no problems. The mounting and support frames in this equipment section, as in the rest of the pilot plant, were designed by LSU personnel and constructed by Mississippi Test Facility (MTF) support personnel. Virtually all equipment and associated frames in this section were constructed from carbon steel.

Cellulose Treatment Section

The ground cellulose output of the handling section was fed into the slurry tank (Figure 7) by the vibratory feeder where it underwent alkali contacting. The slurry tank is a 60-gal, 304 stainless steel, cylindrical vessel equipped with a propeller-type agitator, an automatic temperature control system (ambient to boiling), and a liquid-level control that activates a solenoid valve to admit makeup alkali solution. The heat for the temperature control system is generated by three 3,300-watt electric heating bands around the outside of the vessel under a 2-in. layer of insulation. The tank is slightly elevated by three legs to make room for the outlet plumbing. The outlet is 1-in. pipe located at bottom center of the tank.

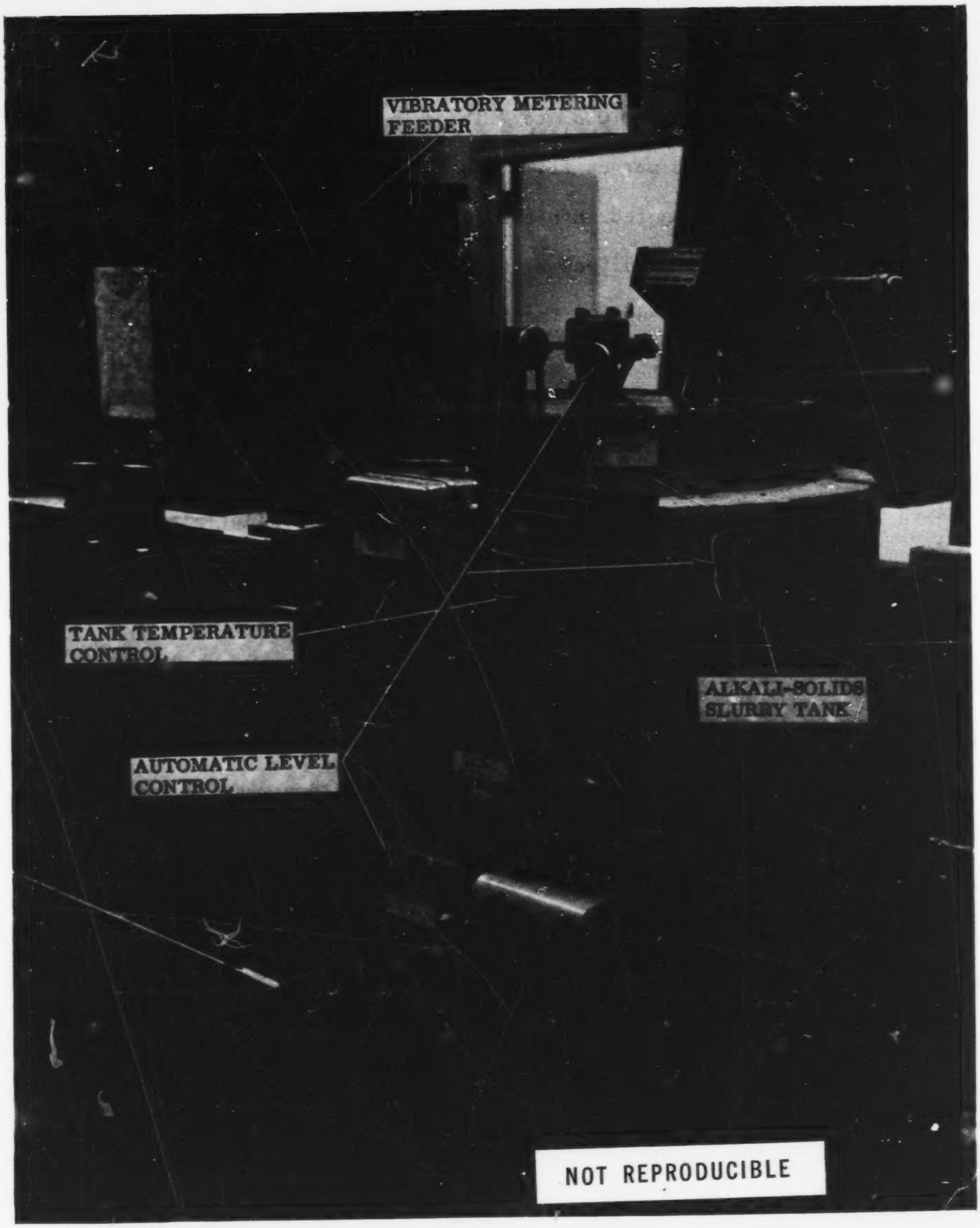

VIBRATORY METERING FEEDER
TANK TEMPERATURE CONTROL
ALKALI-SOLIDS SLURRY TANK
AUTOMATIC LEVEL CONTROL
NOT REPRODUCIBLE

Figure 7. Alkali-solids slurry tank.

The makeup alkali solution is supplied by a 500-gal elevated storage tank. The alkali solution flows through an outlet on the tank to the liquid-level-control solenoid valve and into the slurry tank by gravity.

The contents in the slurry tank are continuously pumped out during cellulosic treatment by a 1/2-hp stainless steel centrifugal pump. From this pump the slurry stream may take one of several routes (indicated as Routes 1, 2, and 3) through the rest of the cellulose treatment section (Figure 8).

Route Number 1. From the slurry tank pump, the slurry flows to the solid-liquid separator (Figure 9) where the cellulose is partially de-watered. It consists of a pair of rubber-coated squeeze rollers, between which a 10-in.-wide monel screen belt passes; an idler roller that holds tension on the belt; and a catch pan that catches the alkali solution squeezed from the cellulose by the squeeze rollers (Figure 9). The tension on the belt is adjustable by increasing or decreasing the spring pressure on the idler roller shaft. The pressure between the squeeze rollers is also adjustable by varying the spring pressure on the shaft of the top roller. The alkali solution is recycled to the slurry tank by way of the catch pan and gravity flow. The separator is also equipped with a spring-loaded scraper that removes the de-watered cellulose from the belt. The lower squeeze roller is driven by the drive system associated with the oxidation oven through a chain and sprocket system. All parts of the separator are stainless steel with the exception of the monel screen belt.

The de-watered cellulose drops from the separator scraper onto the oxidation oven belt (Figure 10). The cellulose is carried through the oven by means of a 12-in.-wide monel screen belt. The belt rides on a drive roller and an idler roller. The drive roller is connected to a 1/6-hp DC variable-speed, electric motor through a gear reduction box and a chain and

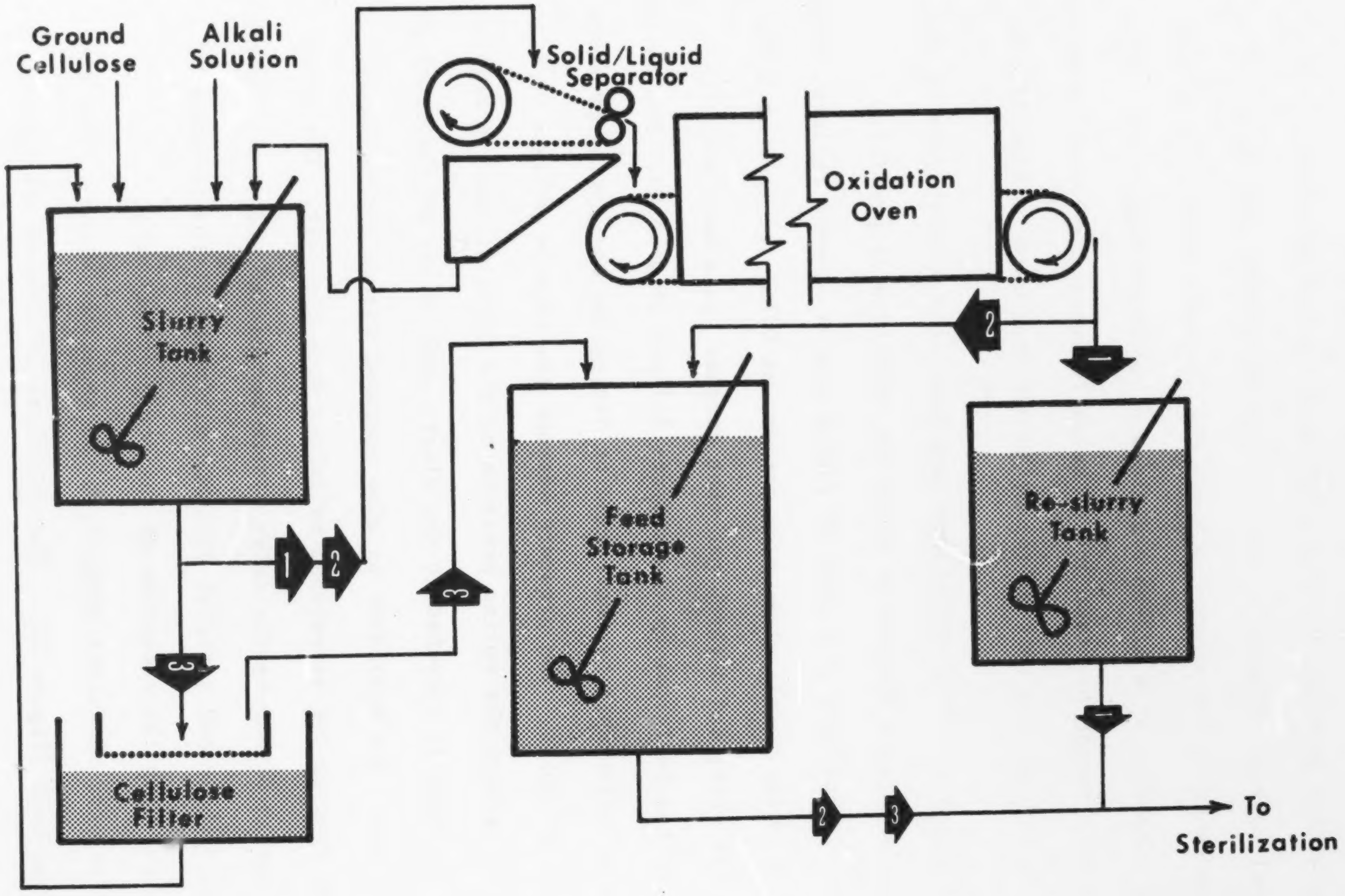
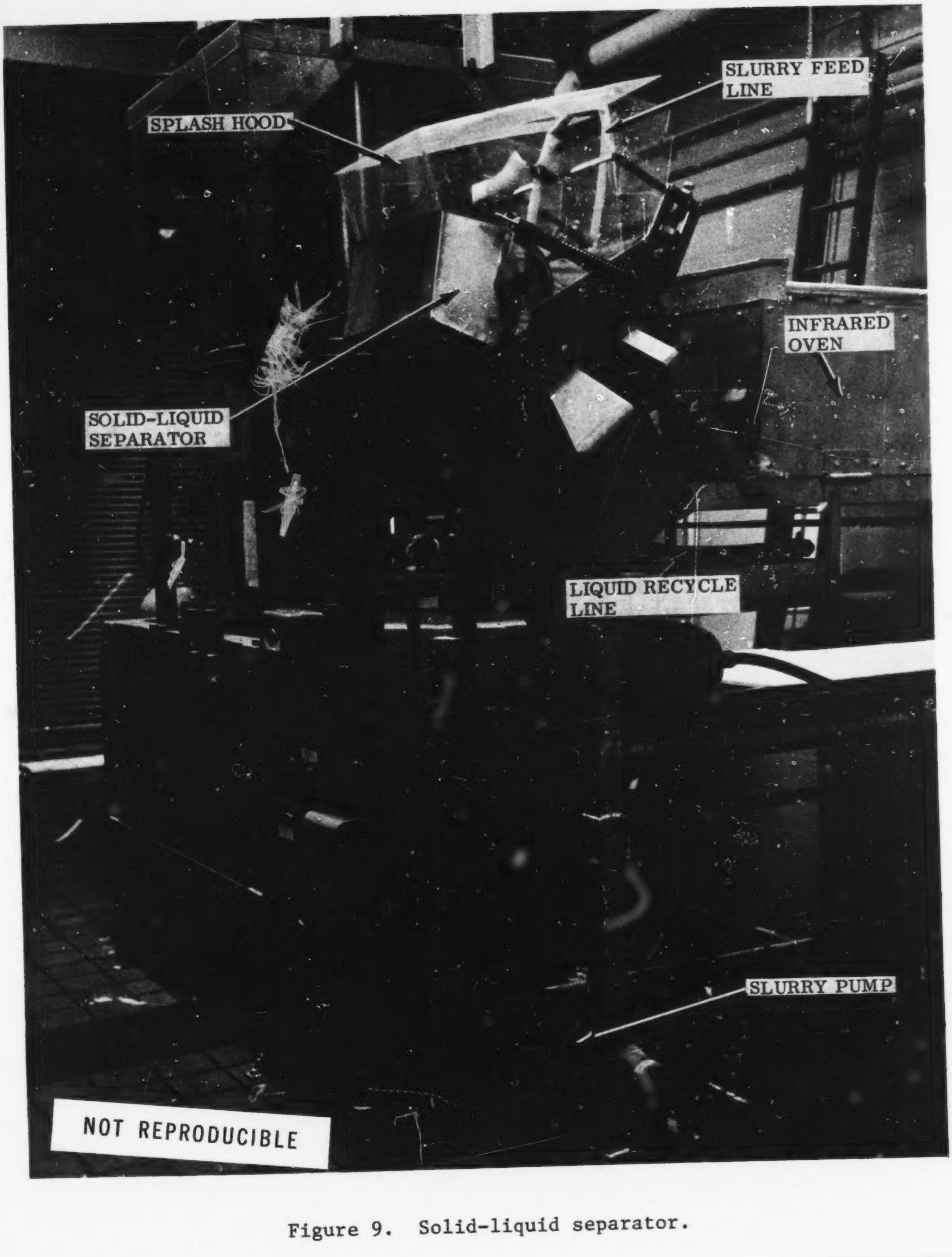

Ground Cellulose
Alkali Solution
Slurry Tank
Solid/Liquid Separator
Oxidation Oven
Feed Storage Tank
Re-slurry Tank
Cellulose Filter
1
2
3
To Sterilization

Figure 8. Cellulose treatment section (numbered arrows indicate alternate routes).


SPLASH HOOD
SLURRY FEED LINE
INFRARED OVEN
SOLID-LIQUID SEPARATOR
LIQUID RECYCLE LINE
SLURRY PUMP

NOT REPRODUCIBLE

Figure 9. Solid-liquid separator.

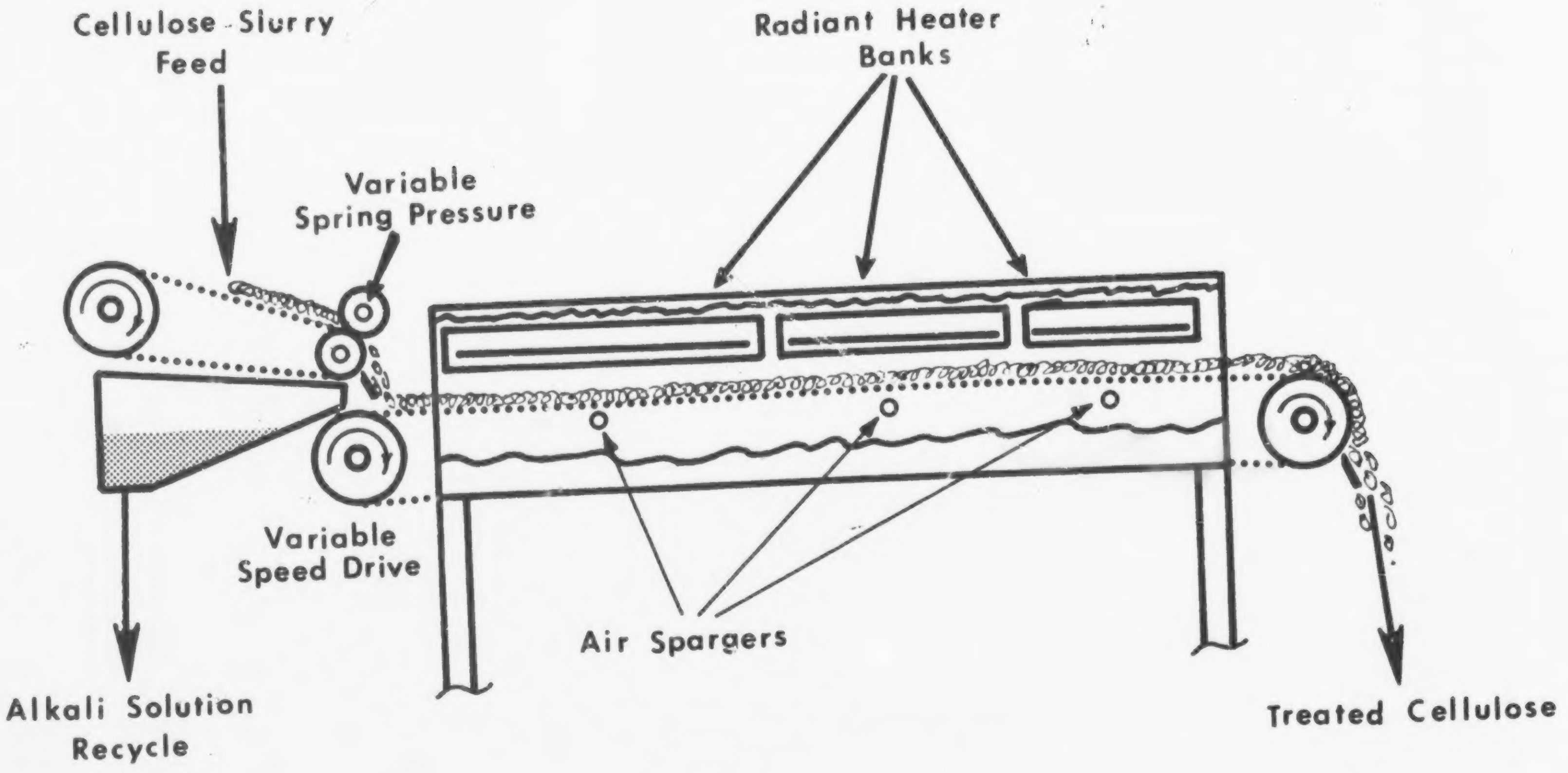

Cellulose Slurry Feed
Radiant Heater Banks
Variable Spring Pressure
Variable Speed Drive
Air Spargers
Alkali Solution Recycle
Treated Cellulose

Figure 10. Solid-liquid separator and oxidation oven.

sprocket. Heat for the oven is provided by three banks of three infrared strip heaters (Figures 10 and 11), which are temperature controlled by time cycling each bank of heaters independently. The heaters in bank one are rated at 5,400 watts. Heaters in banks two and three are rated at 3,300 and 2,400 watts, respectively. The oven is also equipped with an air-sparging system. Air is sparged onto the cellulose from under the screen belt by perforated tubing, and flow is regulated by a rotameter. The oven is equipped with a control panel from which the heater temperatures, the residence time (2 to 12 min) of the cellulose in the oven (belt speed), and the aeration rate are controlled (Figure 12). A scraper is used to remove the cellulose from the belt as was done in the separator. All parts of the oven contacting the cellulose are stainless steel.

As the cellulose continues along Route 1 of the treatment section, it falls from the oven belt scraper into the reslurry tank where it is mixed with de-ionized water and appropriate nutrient salts. This is a 35-gal, cylindrical, steam-jacketed stainless steel vessel (Figure 13). It has an automatic liquid-level control device that adds de-ionized water to a constant level and is agitated by a 1/8-hp, single-propeller agitator. It is temperature controlled by regulating the steam pressure in the steam jacket and is insulated to minimize heat losses. This reslurry operation is the final step in the treatment section. The slurry in this vessel is pumped out a 1-in. bottom-centered outlet by the main feed pump and enters the sterilization section.

Route Number 2. This treatment method uses the same equipment that Route 1 uses except for the reslurry tank. Route 1 requires a very slow feed from the slurry tank to the solid-liquid separator. In Route 2, the alkali treatment in the slurry tank and the oxidation treatment in the oven

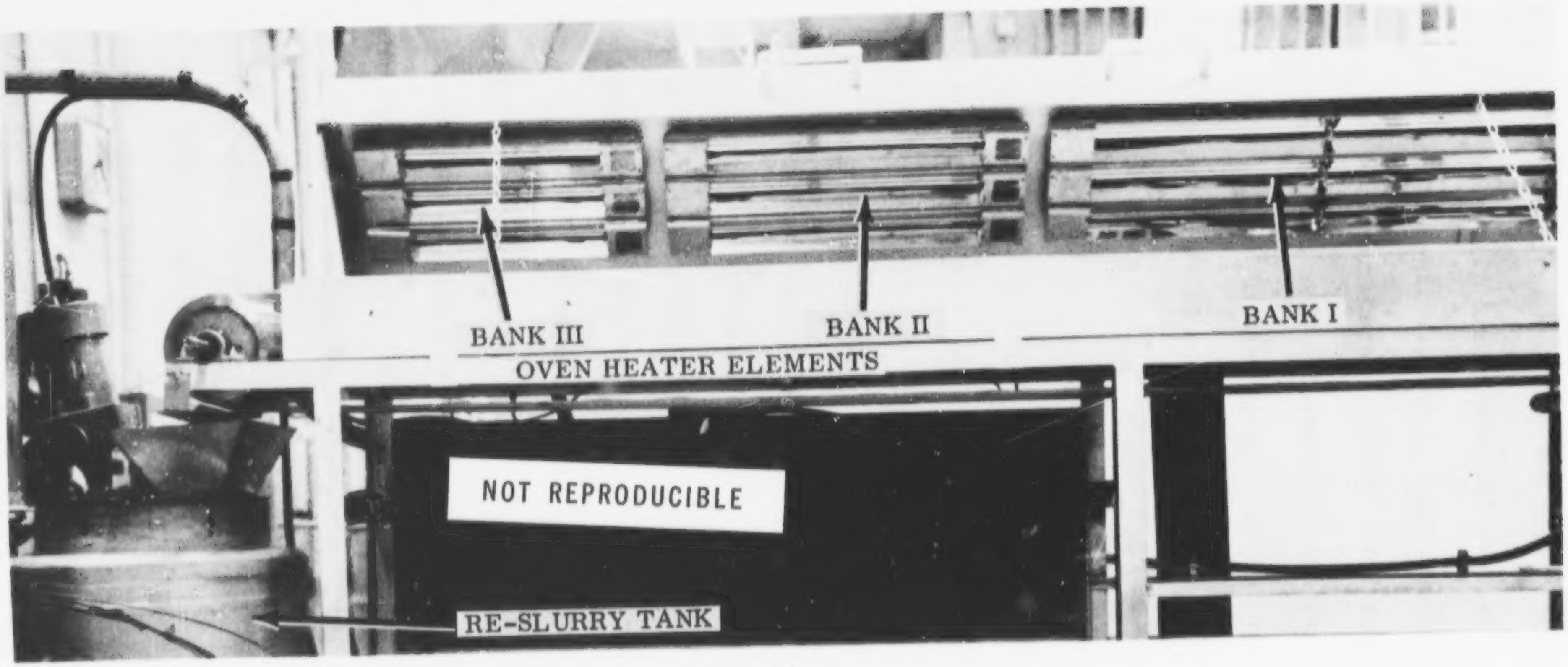
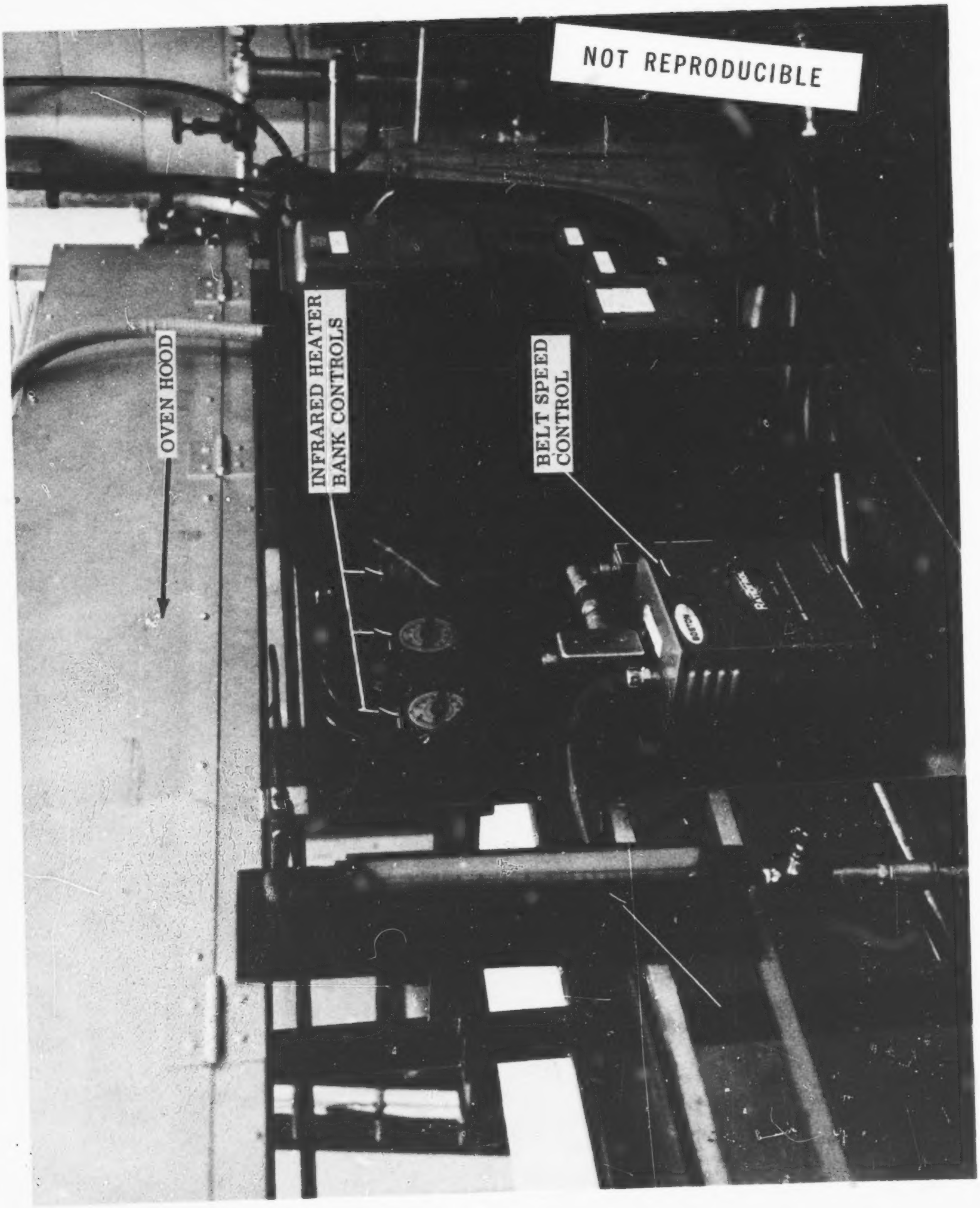
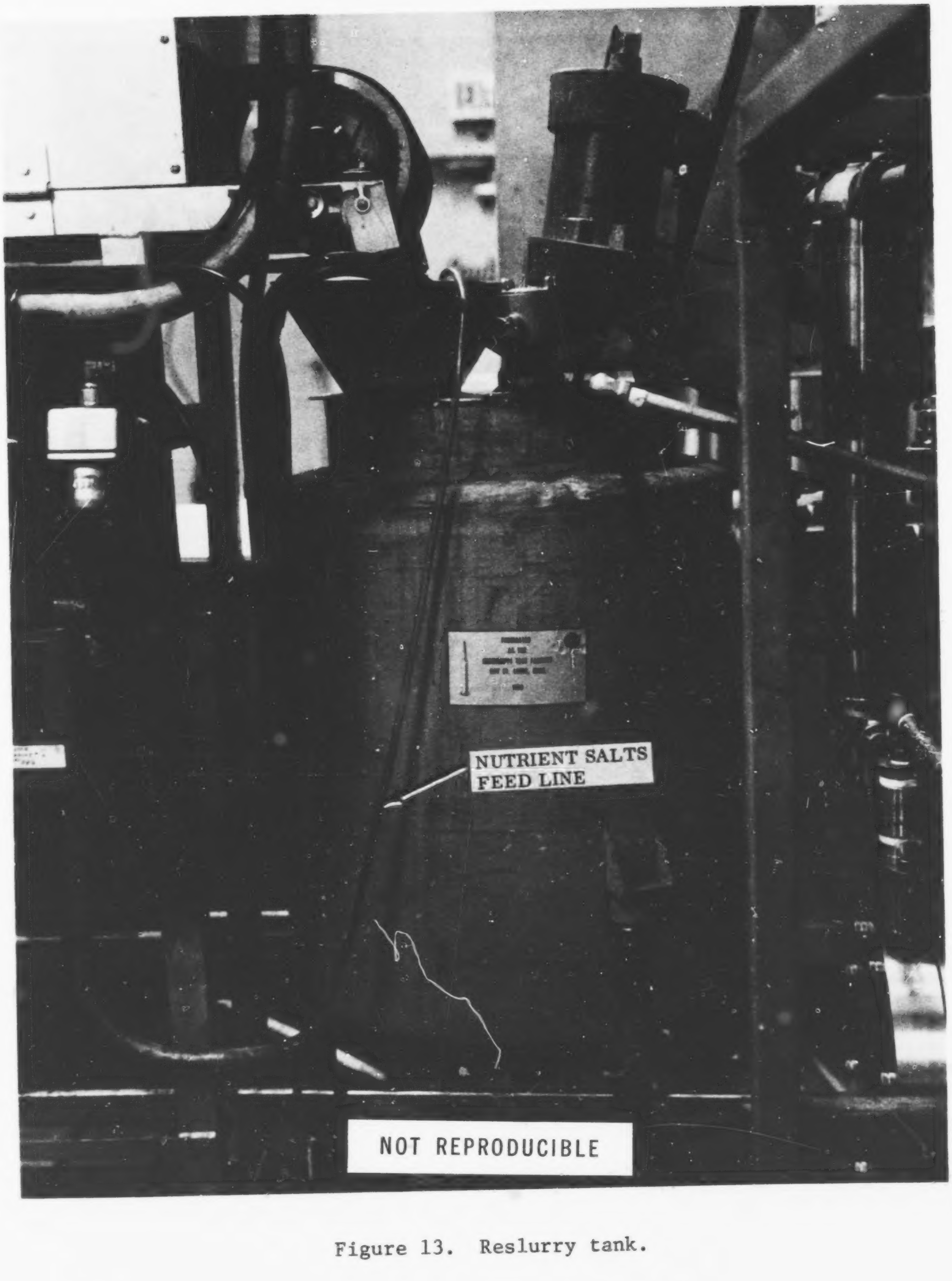

BANK III
BANK II
BANK I
OVEN HEATER ELEMENTS
NOT REPRODUCIBLE
RE-SLURRY TANK

Figure 11. Infrared oven-heating elements


OVEN HOOD
INFRARED HEATER BANK CONTROLS
BELT SPEED CONTROL

NOT REPRODUCIBLE

Figure 12. Infrared oven-control panel.


NUTRIENT SALTS
FEED LINE

NOT REPRODUCIBLE

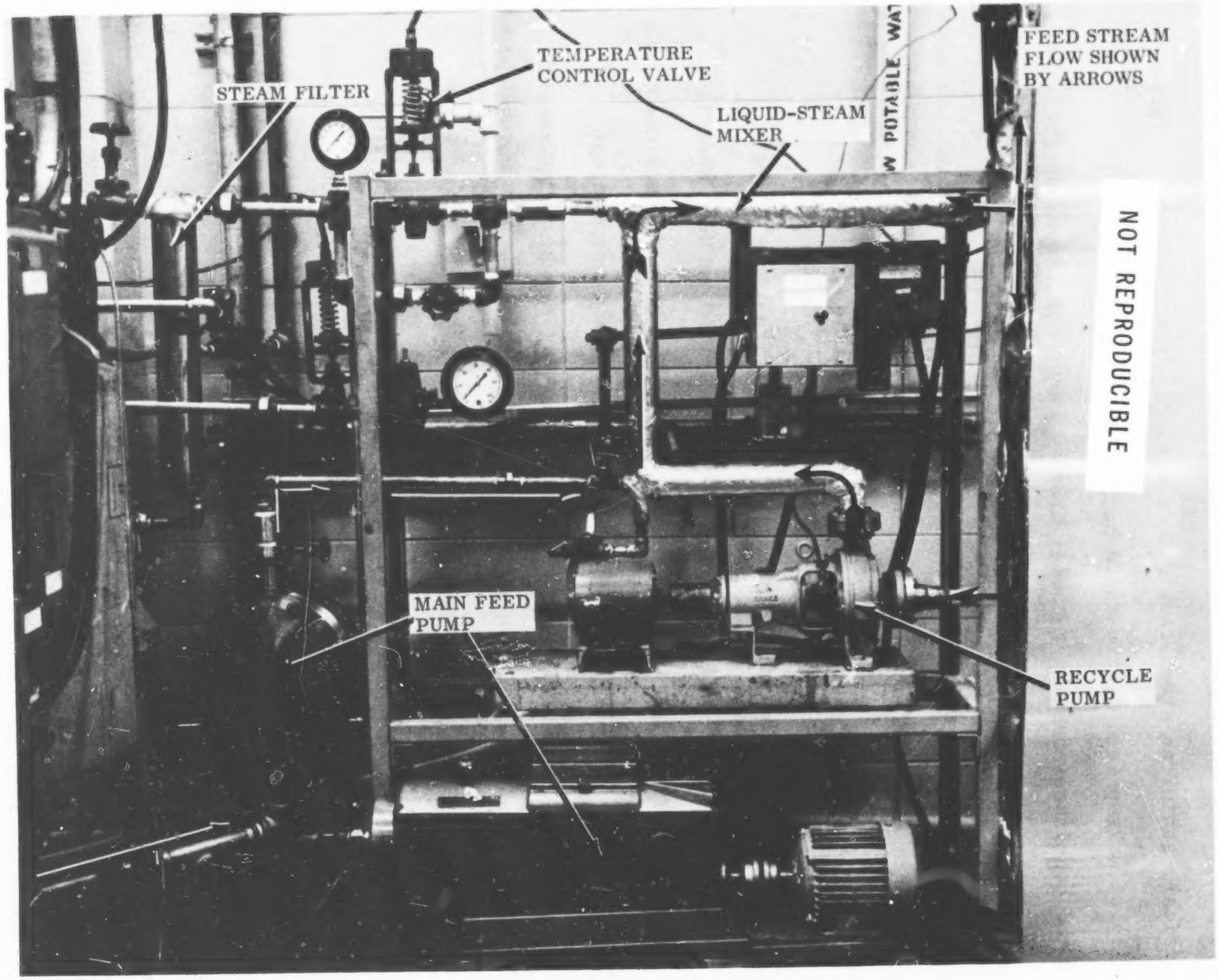
Figure 13. Reslurry tank.

are done at a high feed rate, and the treated cellulose is manually reslurried in the large feed storage tank. This tank is a 500-gal, cylindrical, stainless steel tank with a conical shaped bottom. A large quantity of makeup feed slurry could be prepared in this tank which permitted continuous feeding for many hours without continuous operation of the cellulose-handling and treatment sections. The main feed pump can be supplied directly from this tank instead of the small reslurry tank, as was given in Route 1. A 1/2-hp, single-propeller agitator is used to agitate the feed storage tank.

Route Number 3. The final optional cellulose treatment route is indicated as Route 3 (Figure 8). This treatment method is used when it is not necessary to treat the cellulose in the oxidation oven. The slurry from the slurry tank is fed through a 40-mesh screen filter to remove the cellulose from the alkali solution. The cellulose is manually reslurried in the feed storage tank as in Route 2. The alkali solution is collected in a 2-ft-wide by 3-ft-long by 2-ft-high stainless steel tank. The screen filter fits over the tank and can easily be removed for cleaning. The tank and filter system is mounted on caster wheels and can easily be moved about the pilot plant for other filtering uses. The excess alkali solution is recycled to the slurry tank.

Sterilization Section

The makeup feed slurry in either the reslurry tank or the feed storage tank is pumped continuously into the steam injector system by the main feed pump. This a Lapp Pulsafeeder diaphragm metering pump (Figure 14) and can be set for flow rates ranging from 0 to 2.2 gpm. The fermenter residence time is determined by the feeding rate chosen. The pump can handle slurry


STEAM FILTER
TEMPERATURE CONTROL VALVE
LIQUID-STEAM MIXER
POTABLE WAT
FEED STREAM FLOW SHOWN BY ARROWS
MAIN FEED PUMP
RECYCLE PUMP

NOT REPRODUCIBLE

Figure 14. Steam injector.

densities of up to about 5 percent cellulosic solids by weight without clogging. Care must be taken to prevent clogging of the pump upon starting and stopping the slurry flow through it. Power for the pump is supplied by a 1-hp electric motor.

The main feed pump feeds the slurry into the steam injection system where the feed stream is brought up to sterilization temperature and pressure. The steam injection system (schematic in Figure 15) consists of a temperature-controlled steam injector, a recirculating liquid-steam mixer, and a support stand.

The steam injector injects steam into the liquid-steam mixer loop where it is mixed homogeneously with the feed stream to produce a uniform stream temperature. Steam is metered into the system through an automatic valve that is controlled by a temperature probe located downstream of the steam injector. This valve can be set to produce a feed stream temperature ranging from 260 F to 320 F. There is also a bypass valve that permits manual injection of steam. The inlet steam line is equipped with the necessary filter, pressure relief valve, and trap; a check valve is located between the automatic valve and the steam injector.

The liquid-steam mixer is a recirculation loop constructed of a 1/2-in. stainless steel pipe. The feed stream is pumped through this loop by a 1/2-hp centrifugal pump. The outlet of the liquid-steam mixer is equipped with a 0 to 100 psi bourdon pressure gauge, a copper-constantan thermocouple connected to the 24-point multipoint recorder on the fermenter control panel, a pressure relief valve set at 100 psi, and a 0 to 400 F dial indicating thermometer.

The support stand for the injection system is of carbon steel, unistrut construction. All parts that contact the feed stream are stainless steel.

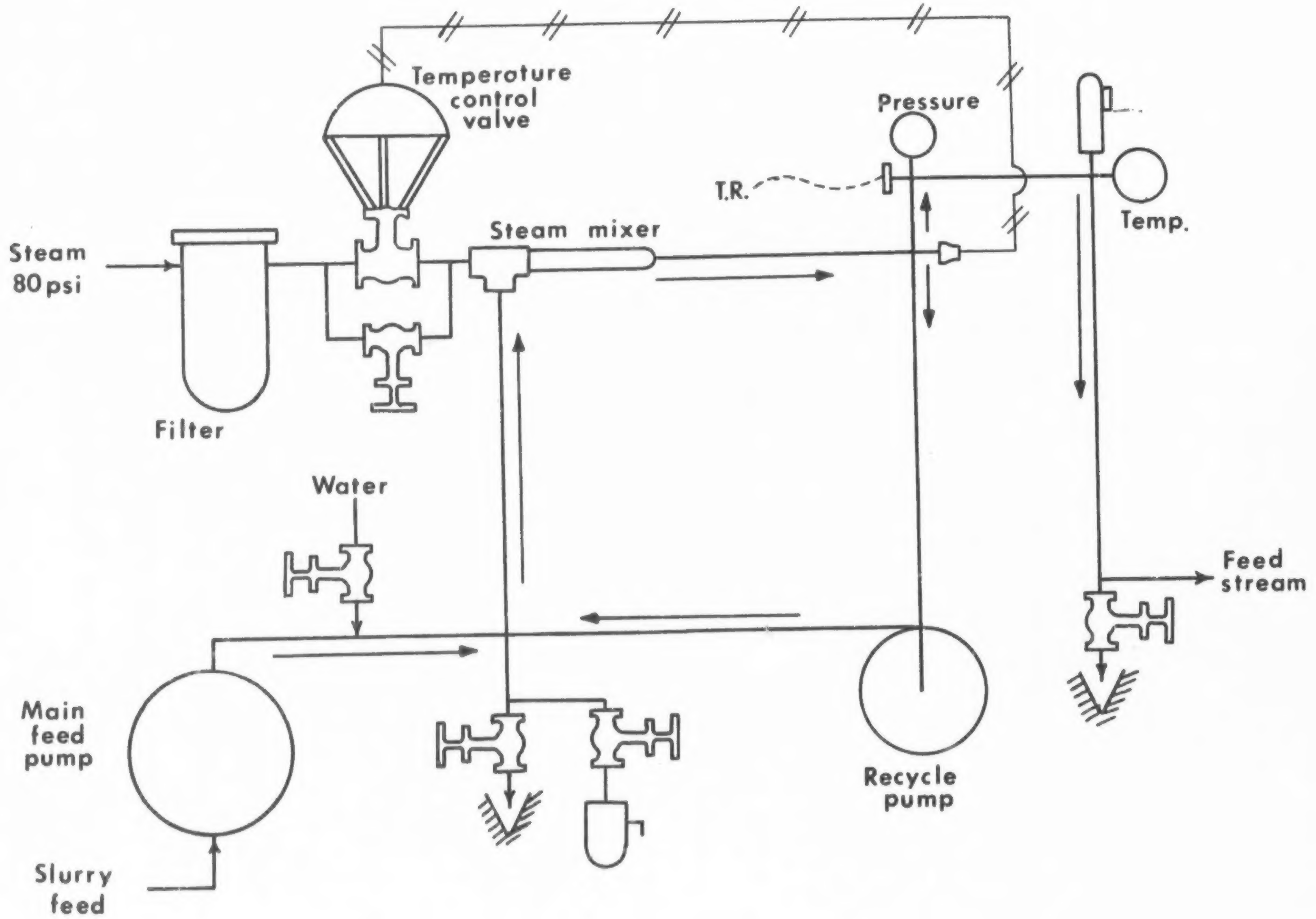

Temperature control valve
Pressure
T.R.
Temp.
Steam 80 psi
Steam mixer
Filter
Water
Feed stream
Main feed pump
Recycle pump
Slurry feed

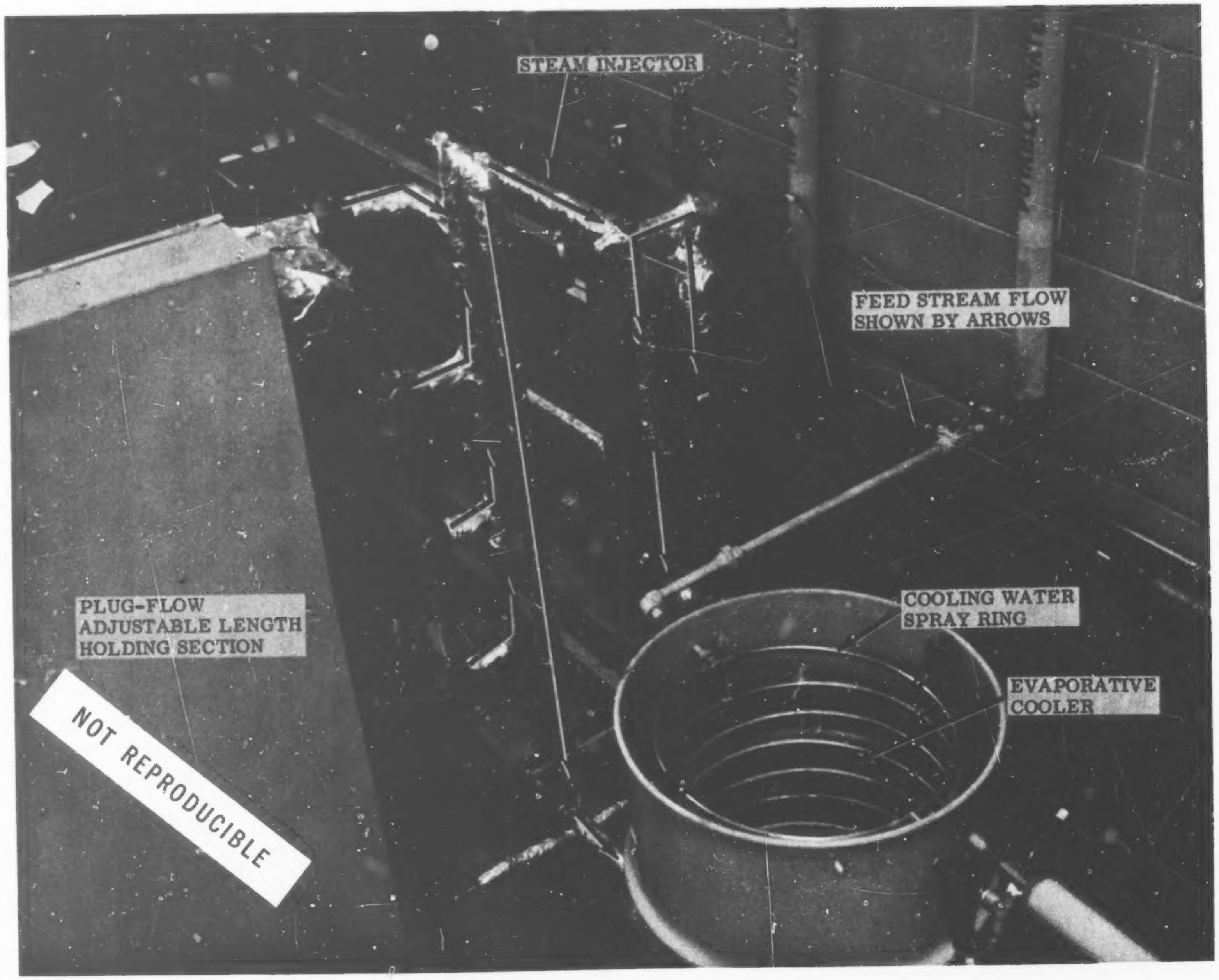
Figure 15. Steam injection heater.

The hot feed stream flows into the sterilizing holding section where the stream is routed in turbulent plug flow through a variable number of insulated tubes (Figure 16). There are four banks of tubes through which the hot feed stream may be routed. These banks provide for four variations in the residence time in the holding section so that a definite temperature-time sterilization sequence can be effected. This variation in residence time is accomplished by the manual switching of four 1-in. three-way plug valves located at the beginning and end of each tube bank. The first tube bank is composed of 22 stainless steel tubes of 1/2-in. diameter that are 10-ft long. Banks two, three, and four each have ten 1-in.-diameter, 10-ft-long stainless steel tubes. All tubing is surrounded by insulation. The outlet of the holding section is equipped with a diaphragm-protected, 100 psi bourdon pressure gauge and a copper-constantan thermocouple attached to the 24-point multipoint temperature recorder.

The final step in the sterilization process is the cooling of the feed stream to a temperature compatible with the contents of the fermenter. This is accomplished in two steps by an evaporative cooler and by a counterflow, double-pipe, chilled-water cooler, respectively (Figures 16 and 17).

The evaporative cooler consists of a coil of 1/2-in. stainless steel tubing positioned in a standard 55-gal drum with a 1/2-in. tube spray ring over the coil (Figure 16). The coil is 18 in. in diameter and contains 10 turns of tubing. The feed stream flows through the coil, and water is trickled over the coils by the spray ring. The drum collects the excess water that flows by gravity to the drain. At low feeding rates, this cooler drops the temperature of the feed stream to approximately 100 F, and the chilled-water cooler is not needed.

The counterflow, double-pipe, chilled-water cooler (Figure 17) is composed of seven 6-ft sections of jacketed tubes attached in series. The


STEAM INJECTOR
FEED STREAM FLOW SHOWN BY ARROWS
PLUG-FLOW ADJUSTABLE LENGTH HOLDING SECTION
COOLING WATER SPRAY RING
EVAPORATIVE COOLER

NOT REPRODUCIBLE

Figure 16. Steam injector, holding section, and evaporative cooler.

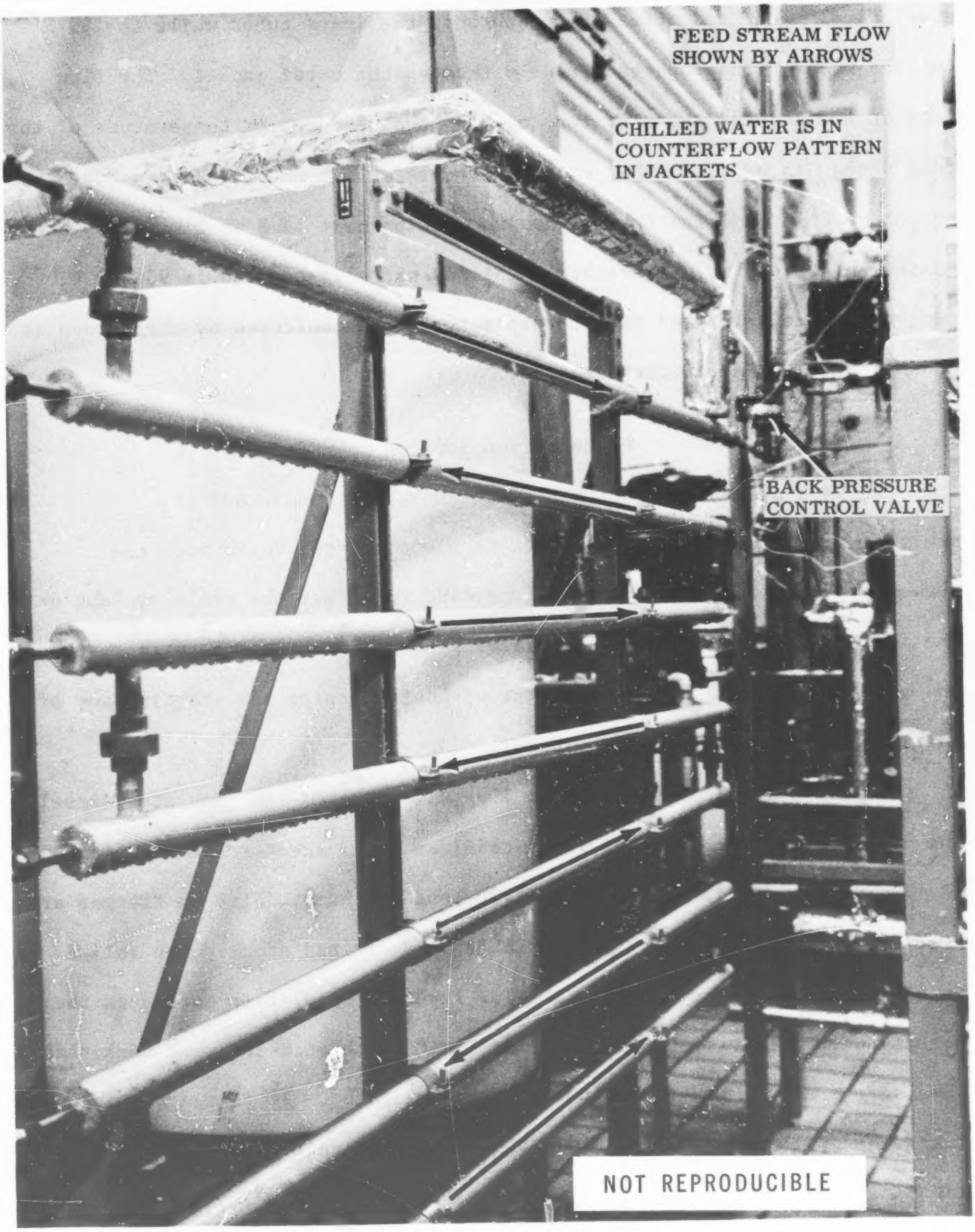

FEED STREAM FLOW
SHOWN BY ARROWS
CHILLED WATER IS IN
COUNTERFLOW PATTERN
IN JACKETS
BACK PRESSURE
CONTROL VALVE
NOT REPRODUCIBLE

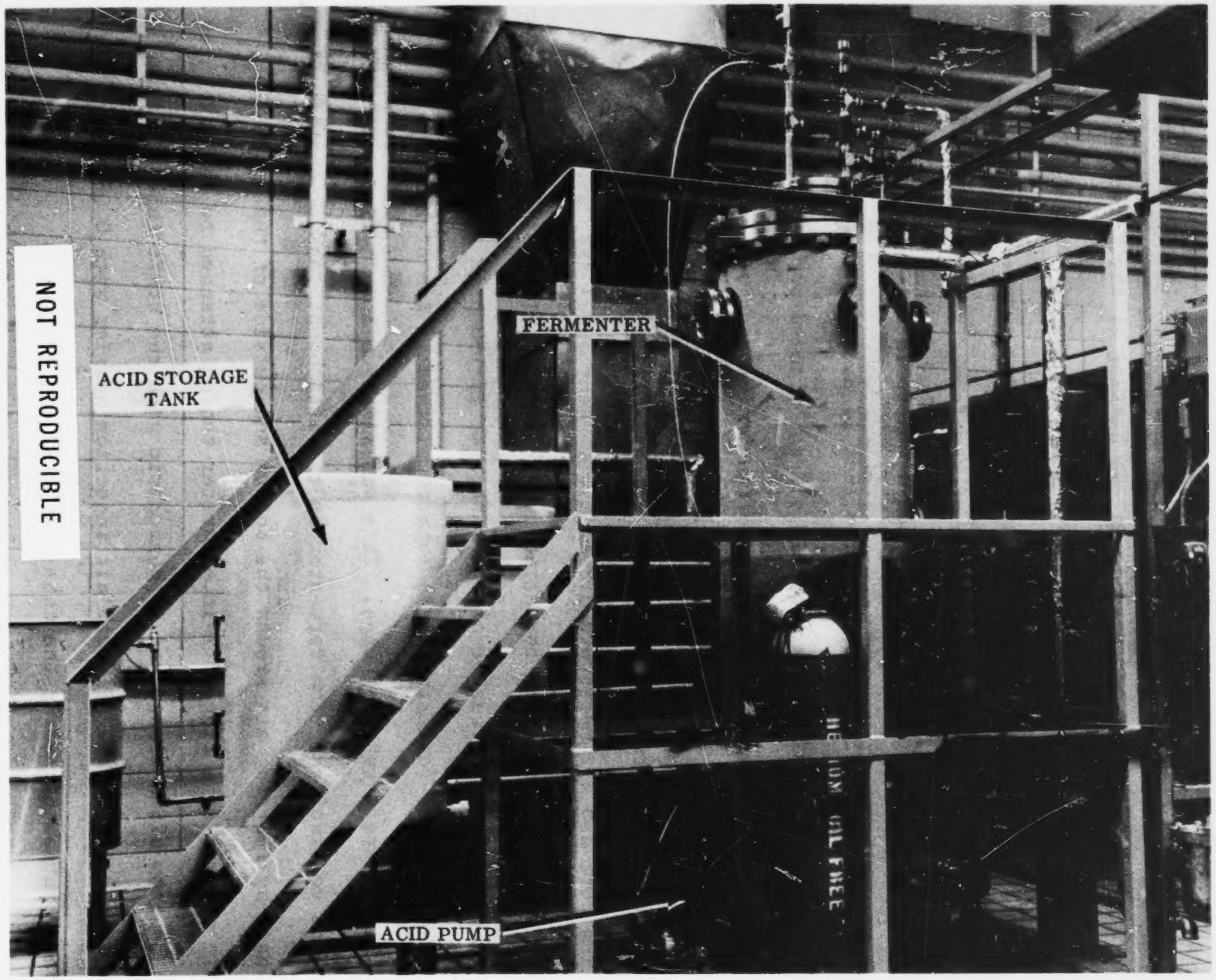
Figure 17. Chilled-water heat exchanger.

inner tubes are 1/2-in. stainless steel and the outer tubes 1 1/4-in. carbon steel pipe. The feed stream flows through the inner tubes while the 50 F chilled water flows countercurrently through the outer jacket. The flow rate of the chilled water is regulated so that the outlet temperature of the feed stream is equal to that of the fermenter. The seven jacketed tubes are supported on a unistrut frame. Maximum design heat load is limited by the maximum heat load on the available chilled-water system and is 90,000 BTU per hour. The inlet and outlet temperatures are monitored by thermocouples connected to the multipoint recorder.

Fermentation Section

The feed stream flows from the chilled-water cooler and the sterilization section through a back-pressure control valve into the fermenter. The feed stream can be recycled to either the reslurry tank or the feed storage tank by a manual valving operation. This permits starting and stopping of feed to the fermenter without stopping and starting any of the feed stream preparation equipment.

The fermenter (Figure 18) is a 150-gal, jacketed and insulated vessel. The inner wall of the fermenter is stainless steel sheet rolled into a 23 1/2-in. inside diameter (ID) by 7-ft-long cylinder. Slip on flanges are used to seal the ends. The outer jacket is stainless steel sheet rolled into a 26 1/4-in. ID cylinder. A 1-in. layer of insulation surrounds the outer jacket. The top blind flange is fitted with an 8-in. weld neck flange that serves as the mounting flange for the agitator. It is also equipped with a 4- by 6-in. hand hole and cover, a 1-in. pipe feed stream inlet and inoculation port, and a 1-in. pipe air vent outlet. The hand hole is used in the initial filling of the fermenter and for cleaning purposes. The


ACID STORAGE TANK
FERMENTER
ACID PUMP

NOT REPRODUCIBLE

Figure 18. Fermenter.

bottom blind flange is fitted with a 2-in. weld neck flange and a 1/2-in. pipe air inlet port. The weld neck flange is the outlet for fermented media. There is a 3/4-in. pipe inlet and an outlet at the top and bottom of the outer jacket. These are used for steam heating of the fermenter contents for initial autoclaving of the fermenter and for temperature control of the fermenter during a fermentation run. There are two 5-in.-diameter view ports near the top of the fermenter internals. At the same level as the center of the view ports, there is a 1-in. pipe inlet through the inner wall of the fermenter that is used for the liquid-level control probe. Opposite this probe is a 2-in. flanged port that is not used at present but was designed into the fermenter for possible future operation of the fermenter in series or parallel with a second fermenter. At mid-height on the fermenter is a 3-in. flanged outlet that is used as a sampling port and a temperature probe inlet. Four small tabs are welded on the inside of the fermenter for attaching internal baffles or draft tubes (Figure 19). Design pressure of the fermenter is 150 psi.

Mixing of the fermenter media is accomplished in one of two ways. Initially, a draft tube was used. This was simply a long cylindrical tube suspended vertically in the fermenter by the attachment tabs. A draft flow is caused by sparging air around the bottom and outside of the draft tube. The air sparger is a ring of 1-in.-diameter tubing attached to the air inlet on the bottom blind flange and is concentric with the draft tube and the walls of the fermenter. Sixteen air injection nozzles with 0.063-in. orifices are positioned on the tube ring.

The agitator is a 3-hp, variable-speed drive with a double mechanical seal, two flat blade turbines, and one pumping turbine at the bottom of the shaft. The rpm ranges from 117 to 300. A steady bearing was attached to the bottom blind flange to support the bottom end of the agitator shaft.

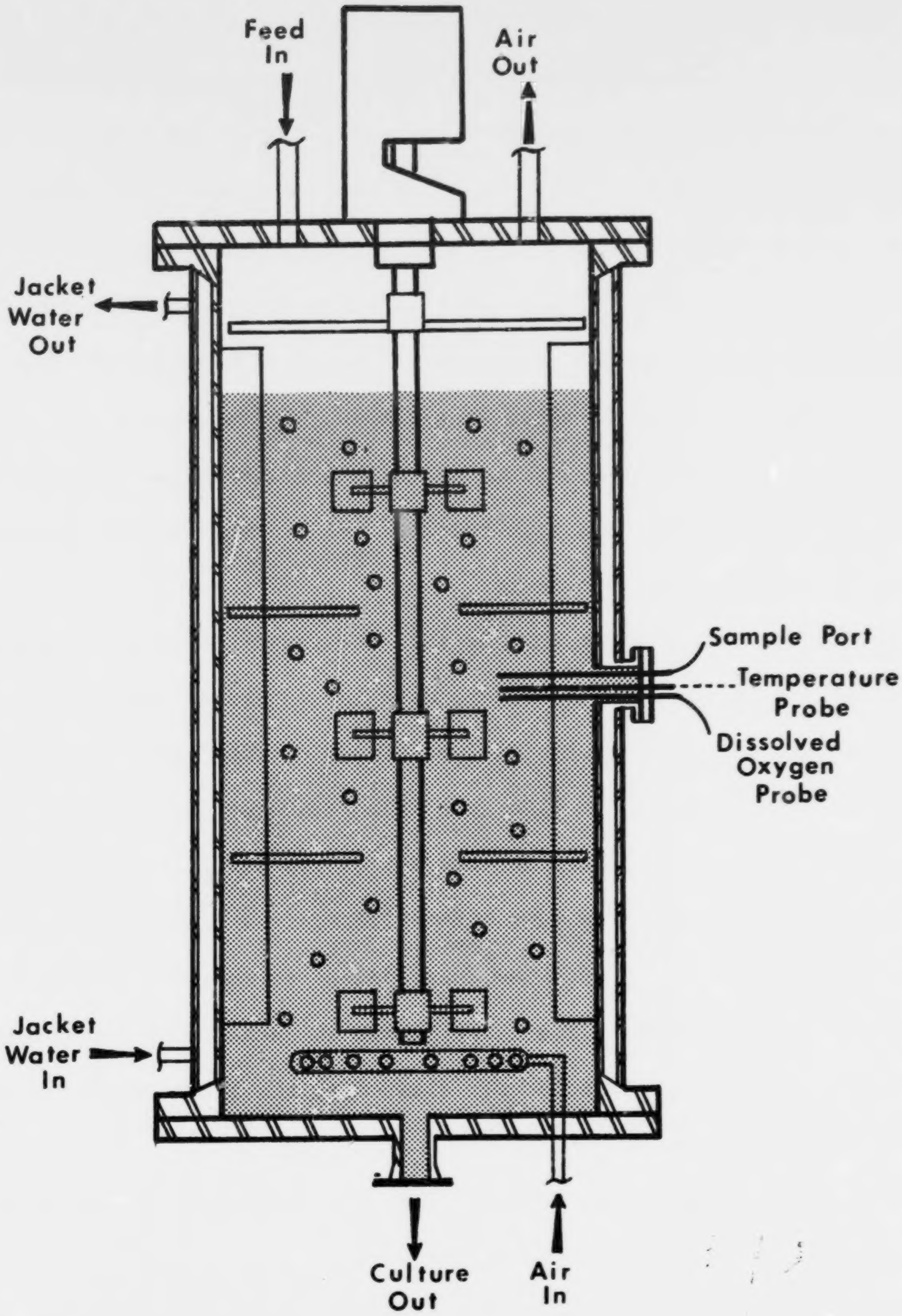

Feed In
Air Out
Jacket Water Out
Sample Port
Temperature Probe
Dissolved Oxygen Probe
Jacket Water In
Culture Out
Air In

Figure 19. Pilot-plant fermenter.

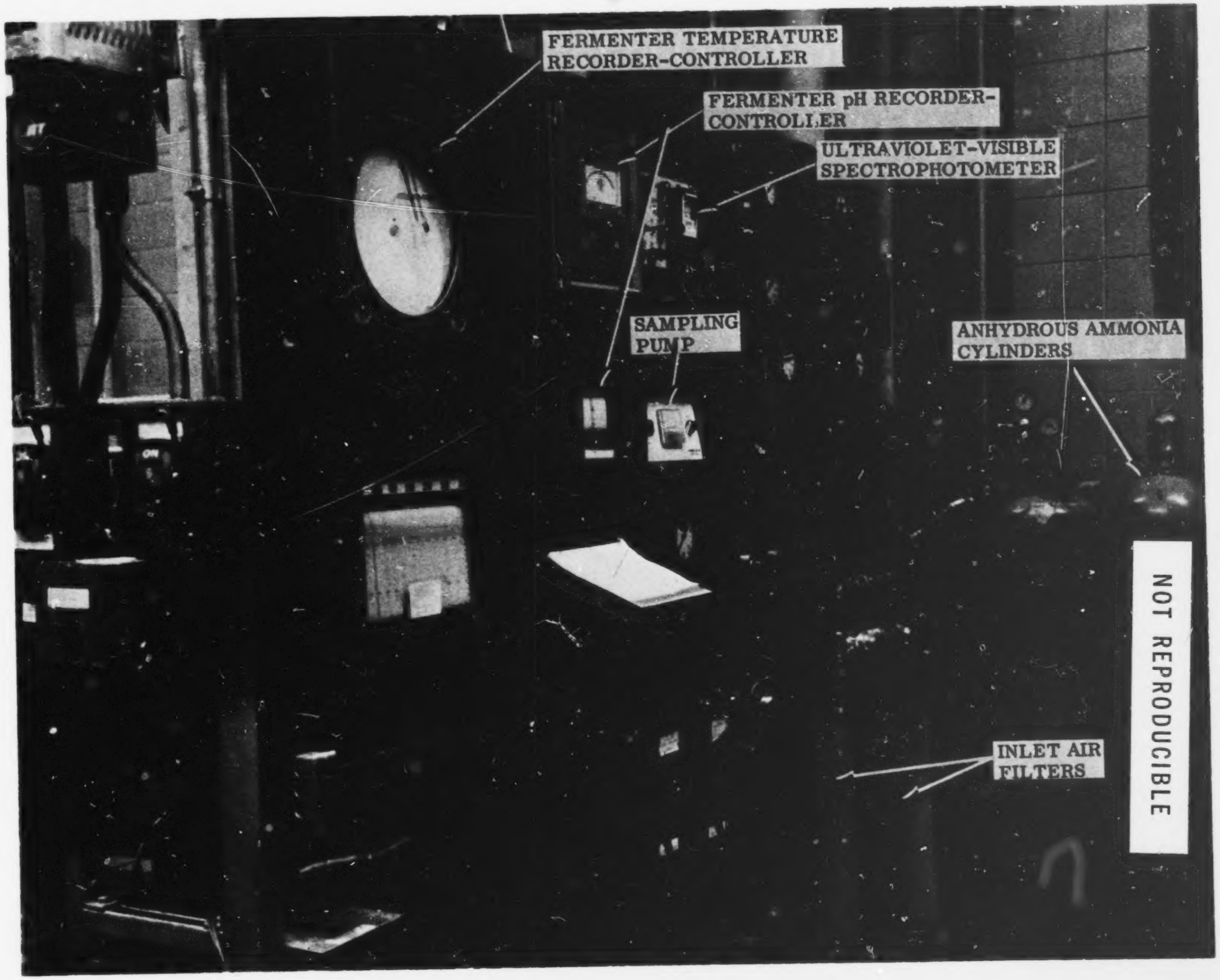
The fermenter is equipped with various controlling and monitoring instrumentation (Figures 20 and 21). The temperature of the media is recorded and controlled by a Honeywell temperature recorder and controller that activates a three-way proportioning valve, which mixes hot and cold water flowing through the outer jacket of the fermenter. The fermenter temperature is also recorded on the multipoint recorder.

The pH of the media is recorded by a strip chart recorder and controlled by a pH meter and controller. The pH is adjusted up when the controller opens a solenoid valve that allows anhydrous ammonia to flow into the inlet air line and is adjusted down when the controller activates a pump that feeds hydrochloric acid into the feed inlet. The flow of anhydrous ammonia is indicated by a rotameter.

The amount of air being sparged into the fermenter media is indicated and controlled by an automatic rotameter. The rotameter regulates a reverse-acting pneumatic control valve. The flow rate range is from 0 to 115 scfm at 0 psig and 60 F. The automatic rotameter is on the outlet air line. The inlet air is passed through one of two air filters in parallel. Only one filter is used at a time. Each filter is a jacketed and insulated tube. Steam is passed through the outer jacket to keep the filter at sterilization temperature. The inner jacket is packed with Dow-Corning extra-fine-tempered glass wool. Air flows into the inner jacket through the top and out the bottom.

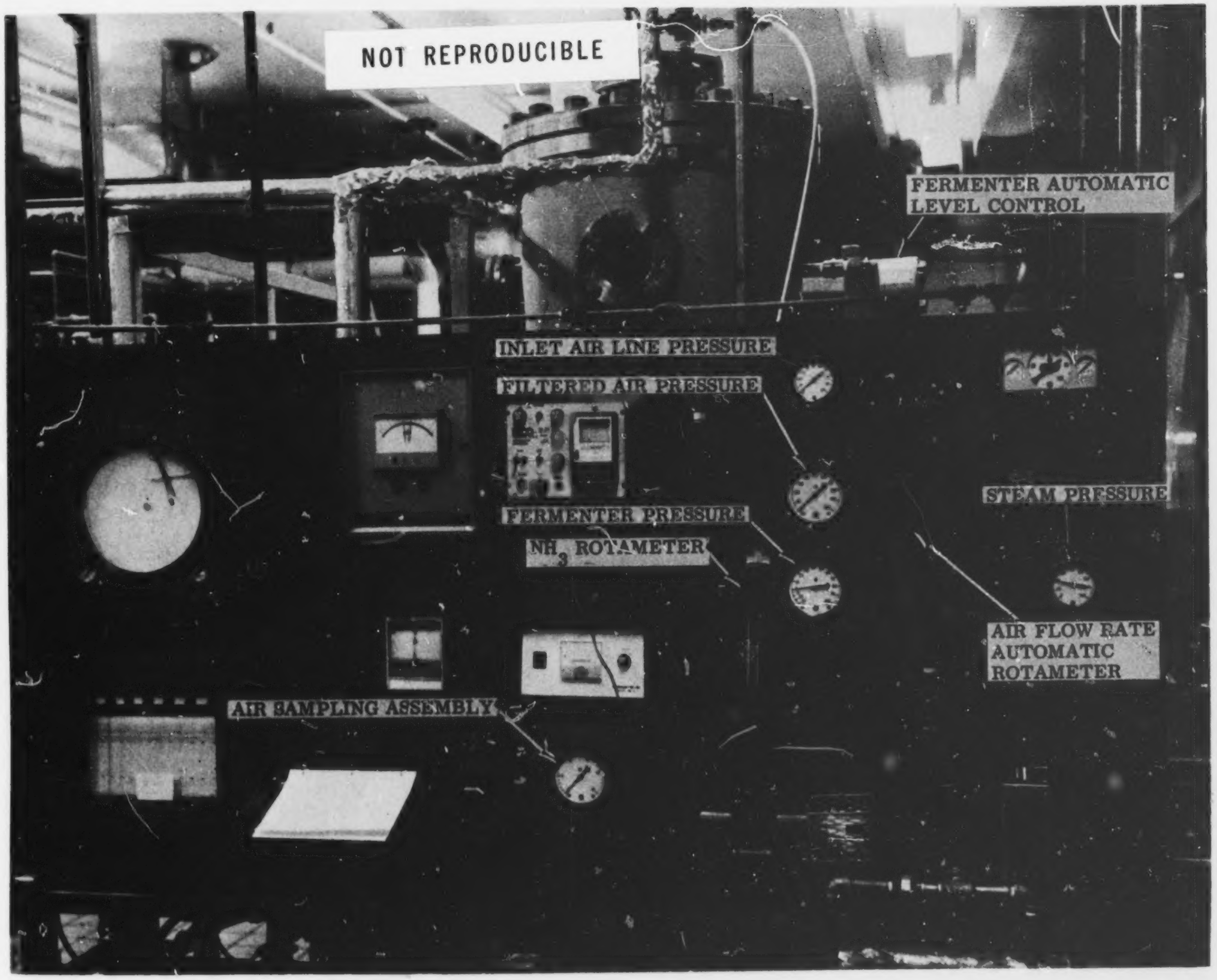
The degree of agitation is indicated by a tachometer and is manually regulated by a hand crank on the agitator.

The fermenter is equipped with a sampling system that, through the use of a positive-displacement sampling pump, provides a sterile seal during sampling. A sampling probe extends into the fermenter approximately 6 in.


FERMENTER TEMPERATURE RECORDER-CONTROLLER
FERMENTER pH RECORDER-CONTROLLER
ULTRAVIOLET-VISIBLE SPECTROPHOTOMETER
SAMPLING PUMP
ANHYDROUS AMMONIA CYLINDERS
INLET AIR FILTERS

NOT REPRODUCIBLE

Figure 20. Control panel--fermenter.


FERMENTER AUTOMATIC LEVEL CONTROL
INLET AIR LINE PRESSURE
FILTERED AIR PRESSURE
STEAM PRESSURE
FERMENTER PRESSURE
NH_3 ROTAMETER
AIR FLOW RATE AUTOMATIC ROTAMETER
AIR SAMPLING ASSEMBLY

NOT REPRODUCIBLE

Figure 21. Control panel--fermenter.

through the sampling port flange. The probe is wrapped with a monel screen shield that helps to prevent clogging of the probe by cellulosic material. The sample of medium is pumped from the fermenter by a nutating disk peristaltic pump. The sample medium is then passed over the pH sensing probe and finally collected for laboratory investigation.

An automatic liquid-level controller on the fermenter maintains a working volume of 140 gal during continuous runs. As the liquid level reaches a predetermined level, the controller opens a pneumatic canister valve on the fermenter outlet line and allows the fermenter media to flow into the harvesting section of the plant.

Power to pH controlling pump, solenoid valves, recorders, and control equipment totals about 1 hp. All material contacting the media in the fermentation section is stainless steel.

Harvesting Section

Several methods are currently used to harvest the cells from the fermented medium. Figure 22 shows the first of these schematically. The medium is dumped into the first 500-gal mixer/settler tank (Figure 23), where the unused cellulose is settled out and drawn off as an underflow stream. The mixer/settler has four outlets at various levels on the tank wall. The residence time of the medium in the settling tank is varied by choosing a particular overflow outlet.

The overflow from the first tank then goes into the pH adjustment tank (Figure 23) where hydrochloric acid is pumped in for pH adjustment. The pH is recorded and controlled by a strip chart recorder/controller. The tank is agitated by a propeller-type agitator.

The overflow from the pH adjustment tank goes into the second mixer/settler tank. This tank is essentially like the first mixer/settler

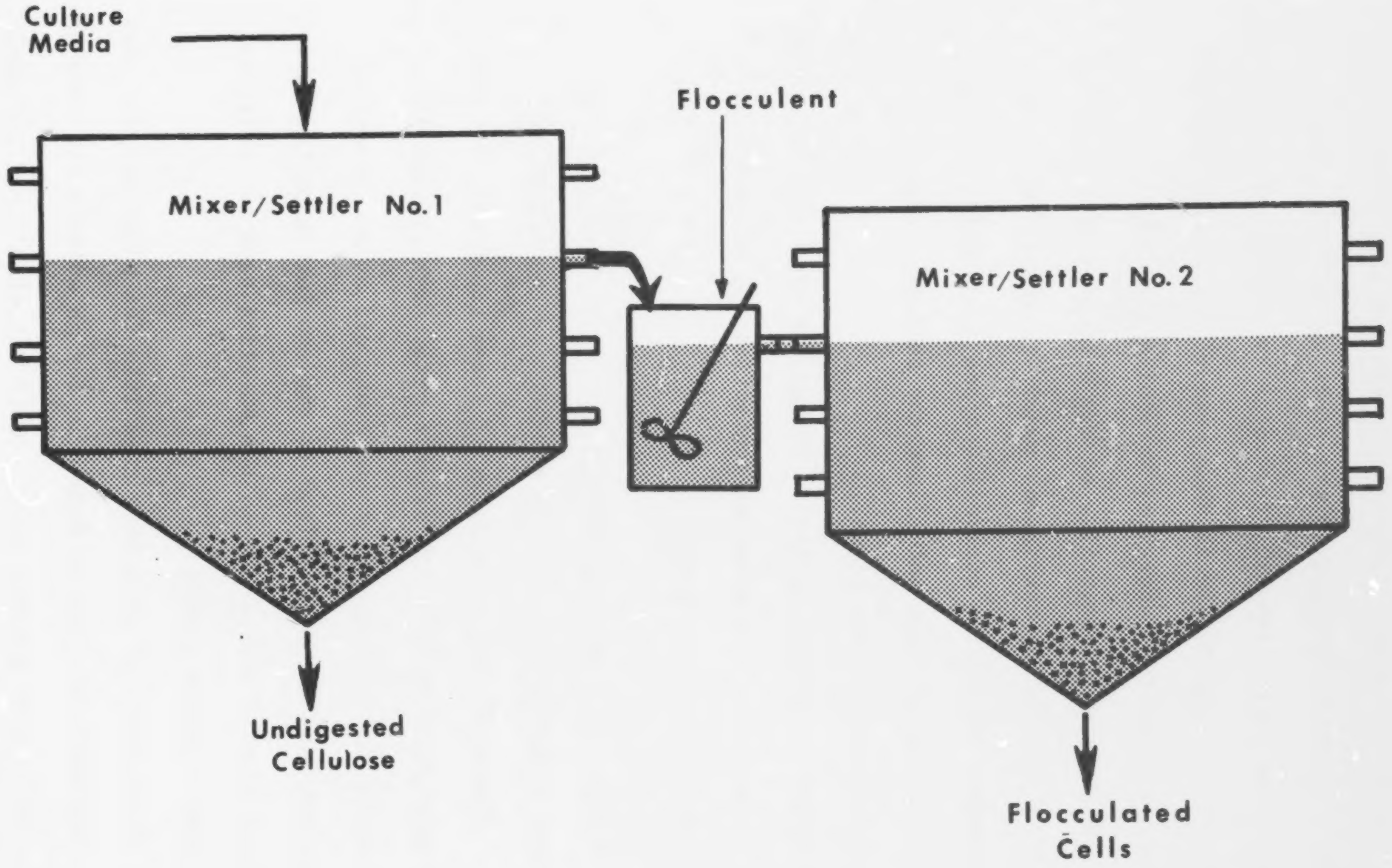

Culture Media
Flocculent
Mixer/Settler No. 1
Mixer/Settler No. 2
Undigested Cellulose
Flocculated Cells

Figure 22. Cellulose and cell concentration.

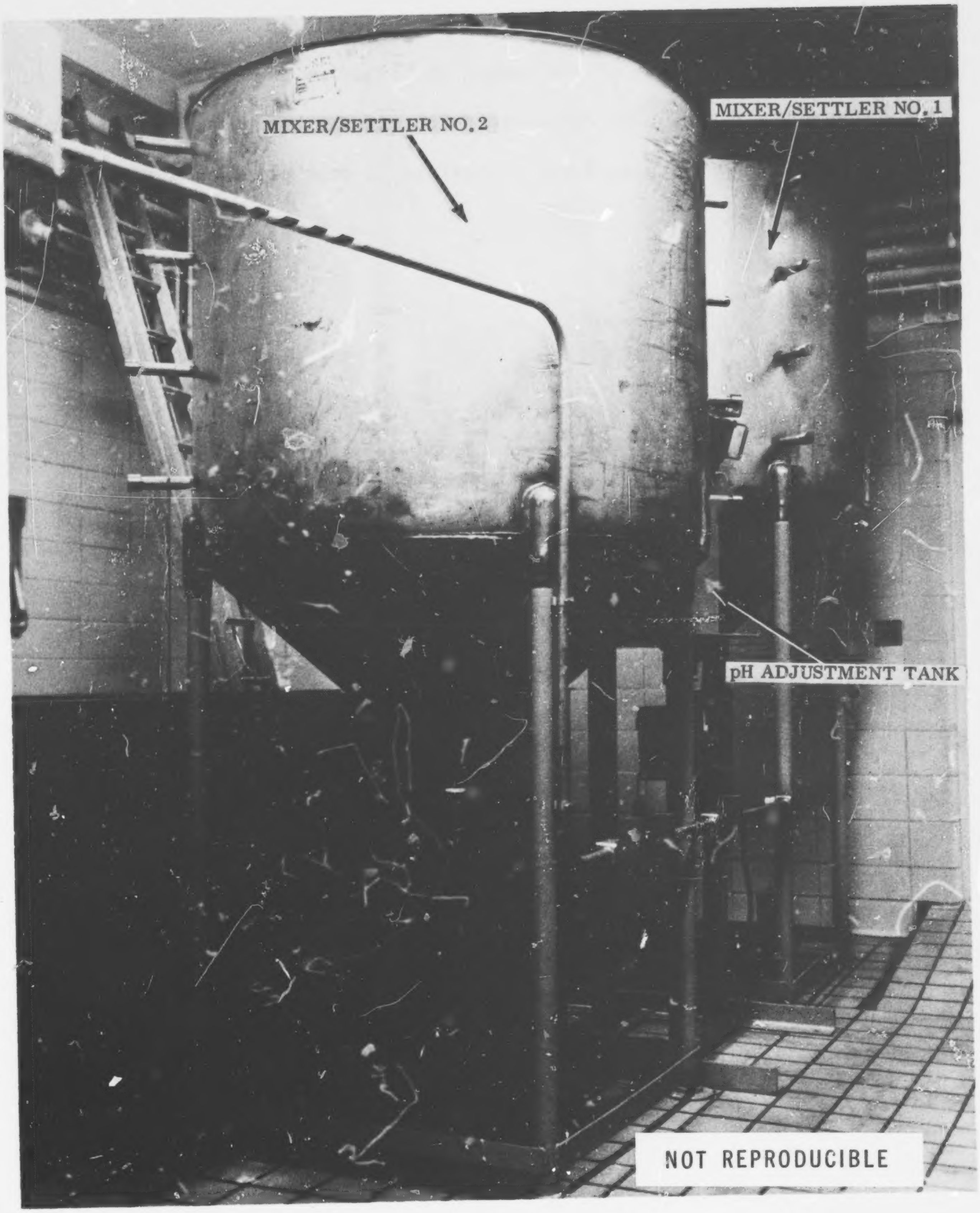

MIXER/SETTLER NO. 2
MIXER/SETTLER NO. 1
pH ADJUSTMENT TANK
NOT REPRODUCIBLE

Figure 23. Mixer/settlers and flocculent tank.

except that the overflow outlets are on both sides of the tank and the legs are a few inches shorter. The acid causes the cells to flocculate and precipitate to the bottom of the tank. The overflow from this tank goes to the drain or to reuse. The cells are taken off the bottom and are either centrifuged in the Sharples Super centrifuge or drum dried on a steam-heated double-drum drier.

PILOT-UNIT OPERATION

The pilot unit could be run as either a batch process or a continuous-flow process. Batch operation required operation of the cellulose-treating equipment until enough treated solids were accumulated to charge the fermenter. The fermenter was then loaded with medium, sterilized, inoculated, allowed to operate for a period of time, and dumped. Cells were harvested by a batch precipitation or by centrifugation.

For continuous operation of the plant, a culture medium was prepared and grown as already described to a point within the logarithmic phase of culture growth. The addition of a continuous-feed stream was started, and the volume of culture medium was regulated by periodic culture withdrawals. The volume of the culture medium in the fermenter was never allowed to vary more than 1 percent above or below the standard working volume. Culture withdrawal cycles were rapid enough and withdrawal volumes were small enough to permit a true continuous-flow scheme of operation. Cells were harvested from the exit stream either by direct centrifugation or by continuous precipitation.

Size Reduction of Solids

The primary purpose of the size reduction step was to make the rough agricultural wastes more homogeneous in size. The mill run bagasse was extremely hard to meter, flow, pump, or mechanically handle in any way without some size reduction. When cut through a 1/8-in. nominal screen, the material could be hoppered and fed without undue problems.

It has been reported that there is a correlation between the size of cellulose particles and rate of enzymatic attack.[33] The effects of particle size do not, however, become apparent until the cellulose is reduced to 100-mesh size or smaller. It is prohibitively expensive to reduce particle size to this level on an industrial scale. A 1/8-in. screen was, therefore, chosen because it would produce a material that could easily be handled and permitted an economical grinding operation.

A rotary knife grinder was chosen as the size reducer primarily because of power costs. A hammer mill has a lower initial cost but uses two to three times as much horsepower per unit of capacity. The knife cutter produces a clean and homogeneous output and is more dust free than an attrition mill such as a hammer mill or shredder.

Solids' Dry Handling

Cellulose particles are fibrous and not free flowing. Their usual bulk density is from 5 to 8 lb per cu ft (at 10 percent moisture). They have a high and irregular angle of repose and tend to bridge and arch badly, especially when their moisture content is more than 15 percent. The solids are noncompressible, and when dry, are rather abrasive to high-speed grinders.

The pilot plant uses a pneumatic conveying system to transfer solids from the grinder to the hopper. Solids are collected in a cyclone and deposited into a vibratory hopper. The vibratory action of the hopper ensures a constant feed to the vibratory metering screw feeder. The vibratory 2-in. screw feeds a continuous stream of relatively constant-mass flow rate to the slurry tank.

When the cellulosic solids become moist or damp, it is extremely difficult to avoid bridging in hoppers and almost impossible to prevent jamming in screw flights or Moyno pumps. The pilot plant's solids-handling system was designed to avoid metering streams that were not either dry solids or light water slurry.

Alkali-oxidation Treatment

Cellulosic solids were contacted with alkali, de-watered, and either contacted with air in an oven or used directly after de-watering.

Solids flowing from the feeder were fed into the alkali slurry tank where they were slurried with sodium hydroxide of from 2 to 4 percent concentration. Trace amounts of an oxidation catalyst (cobaltous chloride) were sometimes added to the slurry. Solids density in the slurry tank was usually held between 4 and 8 percent solids by weight. The tank was agitated, and the temperature was varied from ambient to 160 F. Residence times of the solids in the slurry tank were varied from 30 min to more than 1 hr.

The slurry was pumped continuously from the slurry tank to a de-watering step. If the solids were to receive further oxidative treatment, they were passed through the de-watering squeeze rollers on the oxidation oven. The liquid was returned to the slurry tank. Solids exiting the squeeze rolls retained about 60 percent moisture. These solids were passed through the oven where they were heated and contacted with air. Solids exiting the oven contained from 20 to 40 percent moisture, depending on the severity of the oxidation treatment. Temperature, airflow rate, and residence times in the oven were variable. Surface temperature of the radiant heating elements in the oven was varied between 600 F and 900 F, and residence time of solids in the oven set at from 2 to 6 min. The extent of the oxidation reaction could be controlled in this way, which determined the fraction of cellulose and other carbohydrates degraded to water-soluble products. The cellulosic solids coming out of the oven were dropped in the reslurry or feed stream make up tank. Necessary inorganic salts, antifoam agents, and special nutrients were mixed with the cellulose and make up water in the reslurry tank to prepare the complete fermenter feed stream.

Solids not oxidized in the oven were pumped from the slurry tank into a batch de-watering screen filter. Alkaline liquid was removed and the solids were washed with water to obtain a feed material with a predetermined level of water-soluble carbohydrates. This material was prepared for fermenter feed by batch mixing with inorganic salts, antifoam agents, special nutrients, and water in the 500-gal feed storage tank.

Alkali treatment of sugarcane bagasse is believed to cause depolymerization, decrystallization, delignification, and swelling of the cellulose fiber and thus increases the digestibility of the cellulose by microorganisms. The digestibility of alkali-treated cellulose was reported to be markedly different between different kinds of cellulosics. The effect was said to be considerably more pronounced with hardwoods than with softwoods.[34,35] Thus, the optimum conditions of alkali treatment should be established for each substrate used. Since the direct concern of the contract was the maximum production of SCP, the effect of the severity of alkali treatment on the growth of Cellulomonas has been evaluated.

Finely ground bagasse obtained from the vibratory live-bin hopper in the pilot plant was mixed into various concentrations of alkaline solution (50 g of bagasse per liter of solution) and kept at room temperature with frequent agitation. A portion of the slurry was withdrawn at frequent time intervals and quickly neutralized with hydrochloric acid. The excess liquid was then removed from the cellulose by squeezing the slurry in cheese cloth. One percent wet weight of the treated bagasse was used as a substrate with the basal media already described.

The various media were then inoculated with an equal amount of actively growing Cellulomonas culture and incubated at 30 C on a rotary shaker. The growth rate was determined by measuring the turbidity with a Klett colorimeter. The kinetics of cell growth on the bagasse treated with different concentrations of alkali and different lengths of time showed that alkali treatment of bagasse significantly increased the growth rate and the maximum cell density of the organism. When all bagasse samples were treated for 5 min, the growth rate and maximum cell density were increased proportionally with the increase of alkali concentration (Figure 24).

When the bagasse samples were treated for 2 hr with the same concentrations of alkali, no significant difference was observed among the alkali-treated samples within the range of 0.5 to 10 percent sodium hydroxide concentrations; however, a highly significant difference was noted between the treated samples and an untreated bagasse control (Figure 25).

Since alkali treatment depolymerizes the cellulose polymer, it was suspected that the growth-promoting effect of the alkali treatment was to provide more soluble carbohydrate, which is more readily utilizable by the organism than the insoluble form of cellulose. It was shown that more carbohydrate was indeed solubilized by the alkali treatment (Table 4); however, the level of soluble carbohydrate in the final media was negligible because all the soluble carbohydrate was washed from the bagasse before the media were made. The increase in growth rate of the organism on alkali-treated cellulose was not, therefore, due to providing soluble carbohydrate but to some changes in the insoluble form of cellulose fiber. This inference was confirmed by the result that alkali-treated and thoroughly washed bagasse also supported the growth of the organism as well as alkali-treated, unwashed bagasse.

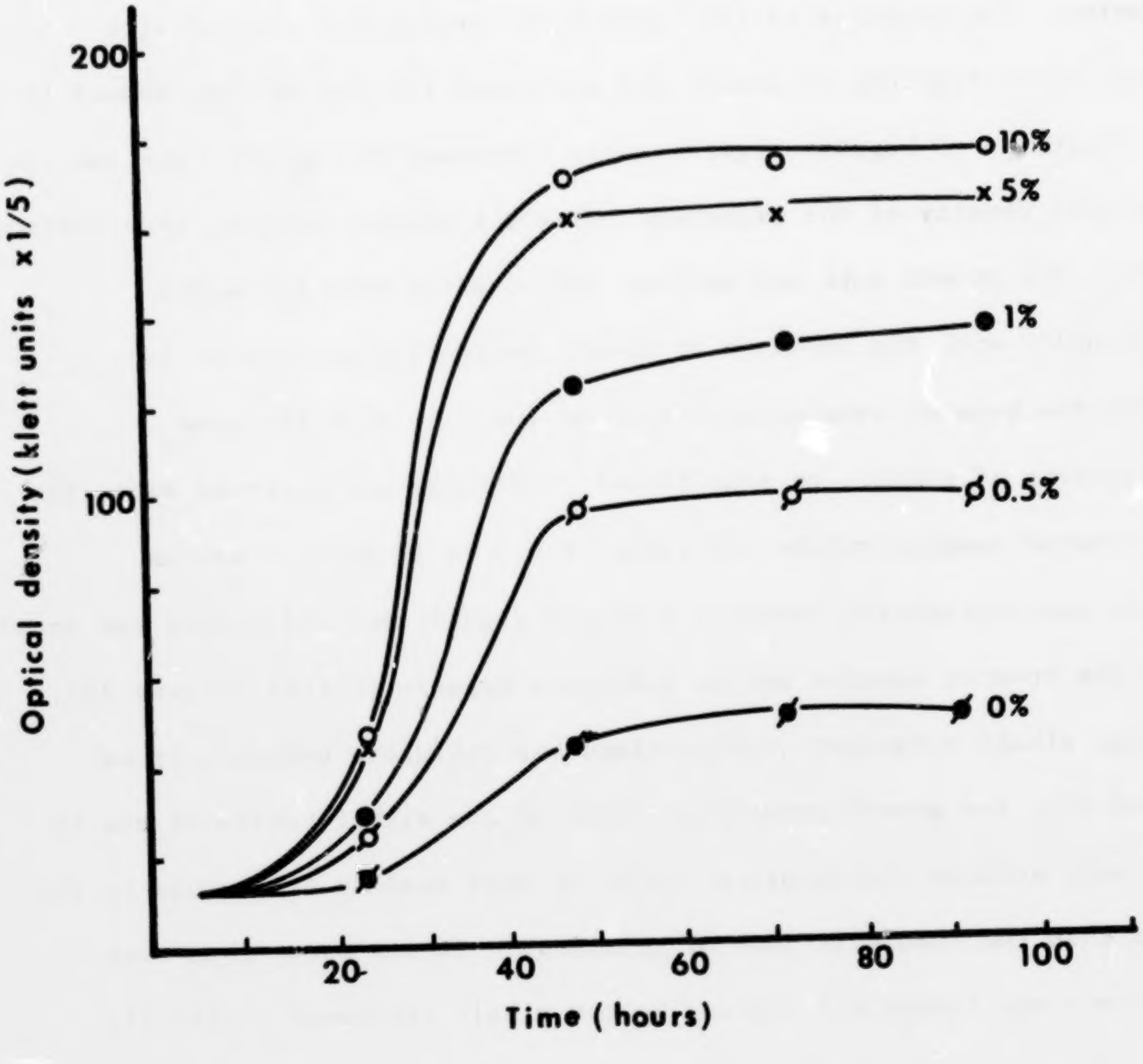


Figure 24. Growth of Cellulomonas on bagasse treated for 5 min. in different alkali concentrations.

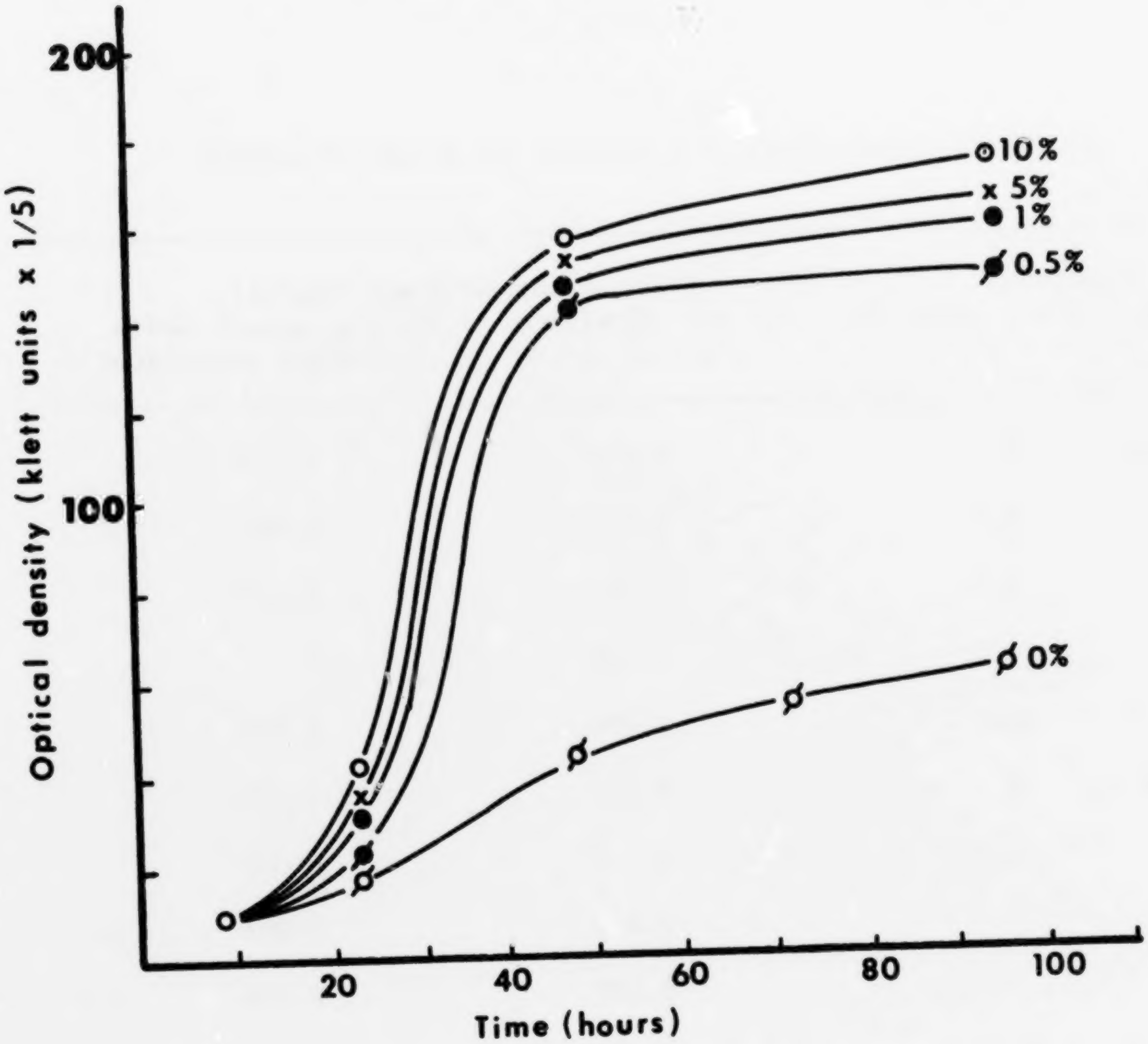


Figure 25. Growth of Cellulomonas on bagasse treated for 2 hr in different alkali concentrations.

TABLE 4

AMOUNT OF CARBOHYDRATE SOLUBILIZED BY ALKALI TREATMENT

Treatment (time, alkali conc, %)		Soluble carbohydrate (mg/ml) In the alkaline slurry	In the growth media (washed substrate)
5 min	0	0.105	0.150
	0.5	0.340	0.008
	1.0	0.360	0.009
	5.0	0.550	0
	10.0	0.480	0.008
30 min	0	0.140	0.160
	0.5	0.370	0.008
	1.0	0.460	0.008
	5.0	0.690	0.008
	10.0	0.630	0.008
2 hr	0	0.105	0.120
	0.5	0.480	0.008
	1.0	0.500	0
	5.0	0.615	0
	10.0	0.580	0.008

Media Composition

The pilot plant culture media were composed of the cellulose source, water, inorganic nutrient salts, trace minerals, special nutrients, and antifoam agents. The original media used the same chemicals as were used for the laboratory shake-flask cultures. The media were changed somewhat for the pilot-plant runs three through five (Table 5).

In order to determine the substrate utilization characteristics of the Cellulomonas organism, shake-flask cultures were prepared with nine different carbon sources (Table 6). All the substrates, except the substituted hydroxyethyl and methyl celluloses, supported growth, lactose and glycerol being the best substrates (Figure 26).

Laboratory tests were run to seek optimum levels of nutrient inorganics. Nine different nitrogen sources were used to find which best supported cell growth. Ammonium bisulfate and ammonium bicarbonate performed better than the rest, and sodium nitrate and ammonium acetate supported no growth at all (Figure 27).

Inorganic nitrogen, in the form of ammonium sulfate, was used at different levels in shake flasks to determine optimum nitrogen level (Table 7). The test showed that the nitrogen level had little effect on initial growth. Ultimately, of course, nitrogen level would cause growth limitation and would have to be maintained at a certain level.

The effects of the level of inorganic phosphate was also checked (Table 8). Optimum levels were seen to be from 0.04 to 0.08 percent phosphorus. In addition to effects on cell density levels, cell growth rate was also affected (Figure 28).

In the original nutrient media used in the isolation of the organism, sodium chloride was included at a level of 6.0 g per liter. Tests have

TABLE 5

PILOT-PLANT MEDIA COMPOSITION FOR RUNS THREE, FOUR AND FIVE

Component		Amount for 1 liter (g)
Substrate:	Treated bagasse (dry weight)	6.0
Nutrients:	Ammonium sulfate	3.0
	Potassium phosphate (dibasic)	0.5
	Potassium phosphate (monobasic)	0.5
	Magnesium sulfate	0.1
	Calcium chloride	0.1
	Sodium chloride	3.0
Other:	Yeast extract	0.3
	Trace minerals*	1.0 ml
	Polyglycol P-2000	0.1 ml
	Water	To 1.0 liter
*Trace minerals composition (g/liter)		
	Calcium chloride	0.5
	Ferric chloride, $6H_2O$	16.7
	Zinc sulfate, $7H_2O$	0.18
	Copper sulfate, $5H_2O$	0.16
	Cobaltous chloride, $6H_2O$	0.18
	Ethylenedinitrilotetraacetic acid	20.1

TABLE 6

EFFECT OF DIFFERENT CARBON SOURCES ON THE GROWTH OF CELLULOMONAS

Carbon source (10 g/liter)	Optical density (Klett unit)	Cell yield[a] (dry wt) (g/liter)
Glycerol	335	0.436
Glucose	250	0.325
Galactose	270	0.351
Cellobiose	195	0.254
Maltose	142	0.185
Lactose	340	0.442
CMC	227	0.290
Methyl cellulose	40	0.052
Hydroxyethyl cellulose	65	0.085

[a] Cellulomonas grown in basal media for 96 hr in shake tubes at 30 C.

shown that less sodium chloride will give much the same growth response (Table 9 and Figure 29). Total exclusion of sodium chloride in pilot-unit fermenter runs produced, however, low cell growth and slow growth rates.

The level of trace mineral solution in the culture media was tested, and optimum level was found to be from 0.5 to 1.0 ml per liter of media (Table 10 and Figure 30).

Since it would not prove industrially feasible to use the laboratory-grade chemicals that had been used in the pilot-plant and laboratory-fermentations, various industrial-grade and fertilizer-grade chemicals were tested to find less expensive replacements for the nitrogen

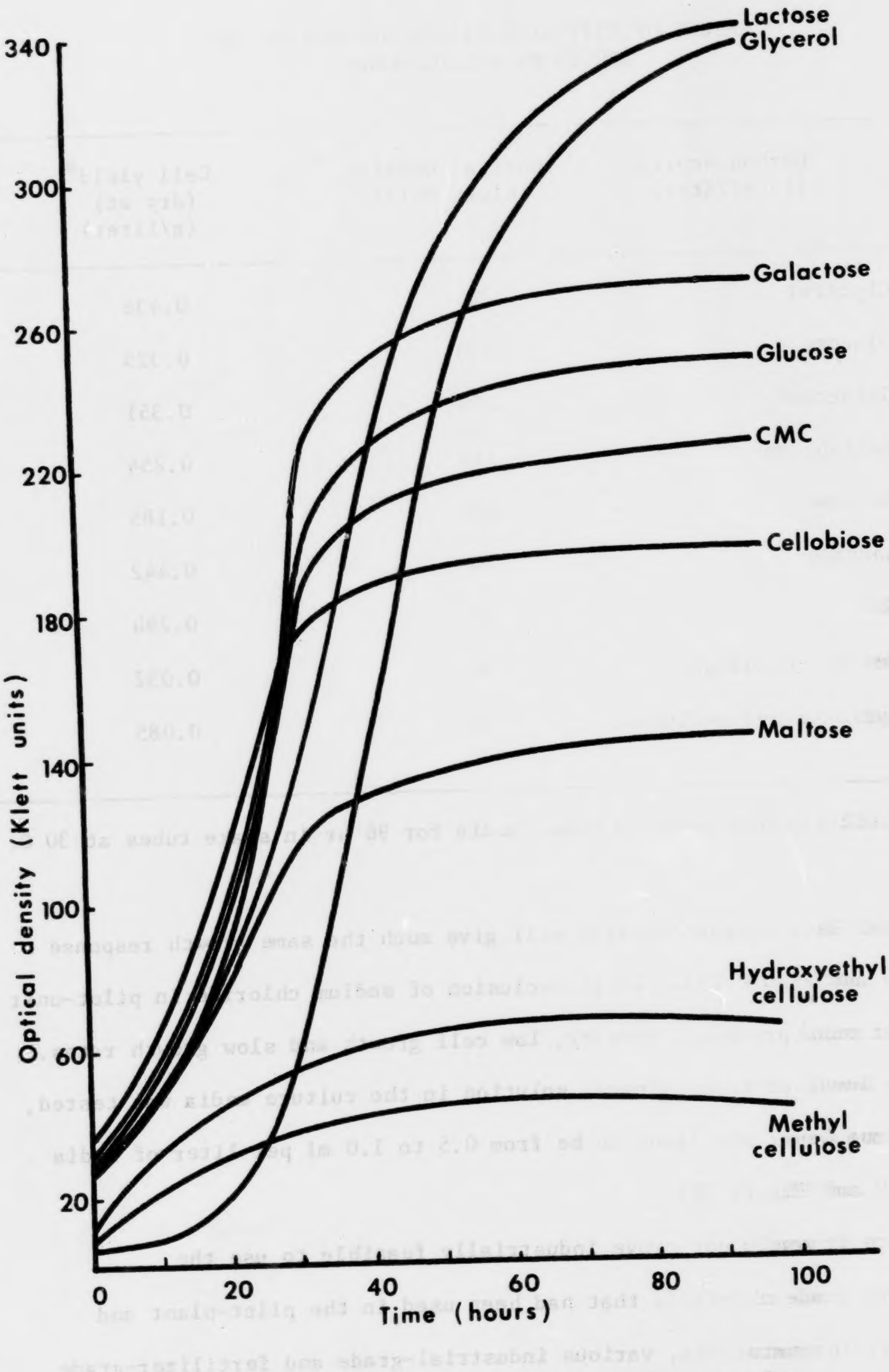
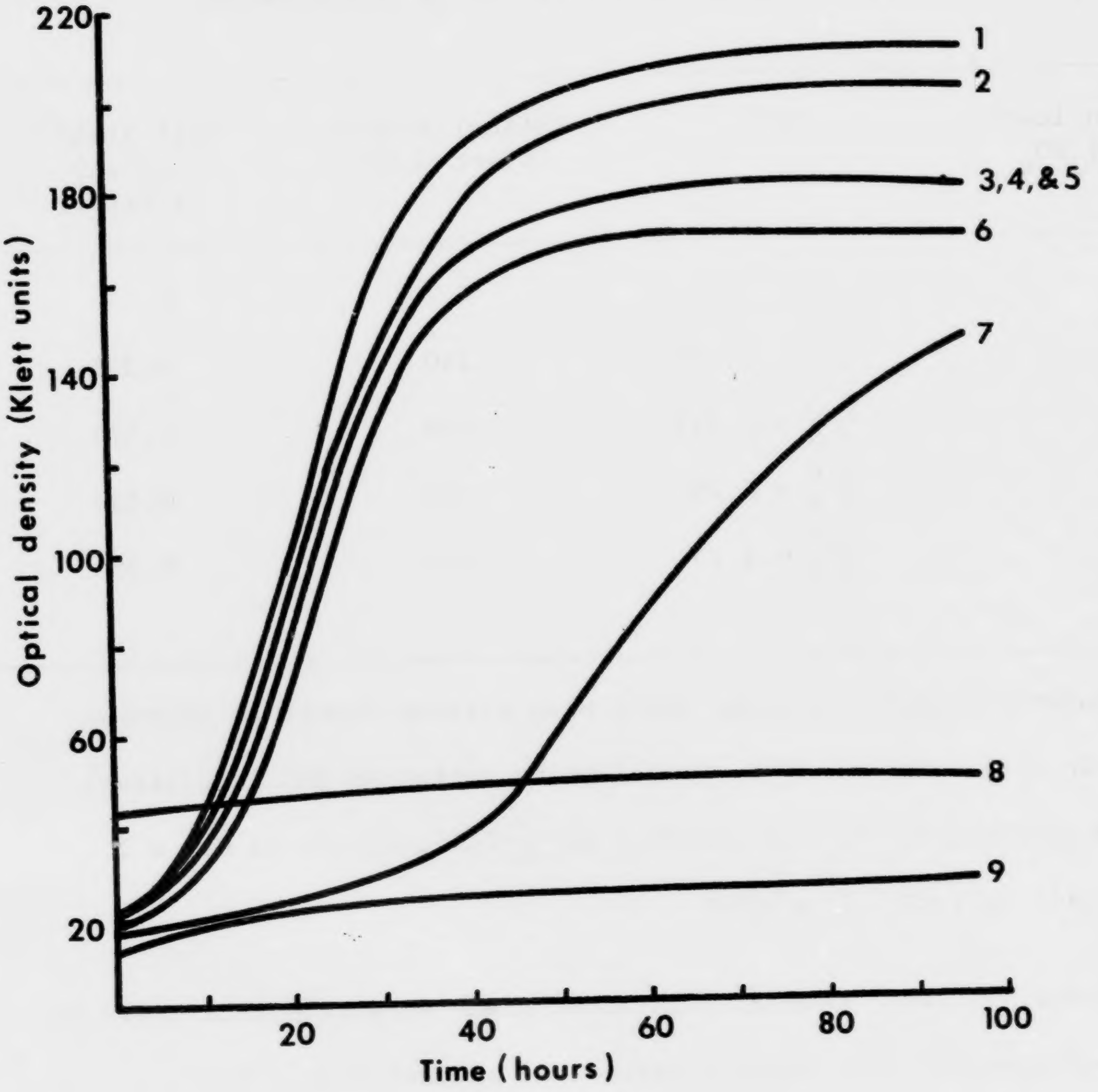


Figure 26. Effect of different carbon sources on the growth of Cellulomonas.



1. AMMONIUM BISULFATE
2. AMMONIUM BICARBONATE
3. AMMONIUM CHLORIDE
4. AMMONIUM NITRATE
5. AMMONIUM SULFITE
6. AMMONIUM SULFATE
7. UREA
8. SODIUM NITRATE
9. AMMONIUM ACETATE

Figure 27. Effect of different nitrogen sources on the growth of Cellulomonas.

TABLE 7

EFFECT OF NITROGEN LEVEL ON THE GROWTH OF CELLULOMONAS

Nitrogen level [% $(NH_4)_2SO_4$]	N/P	Optical density (Klett units)	Cell yield[a] (dry wt) (g/liter)
0		0	0
0.1	$\frac{0.1}{0.8} = 0.125$	260	0.339
0.3	$\frac{0.3}{0.8} = 0.375$	290	0.378
0.6	$\frac{0.6}{0.8} = 0.75$	292	0.380
1.0	$\frac{1.0}{0.8} = 1.25$	272	0.354

[a] Cellulomonas grown in basal media plus various levels of nitrogen for 92 hr in shake tube at 30 C. Basal media contained CMC 10 g/liter; K_2HPO_4 2.0 g/liter; KH_2PO_4 2.0 g/liter; $mgSO_4$ 0.1 g/liter; Ca Cl_2 0.1 g/liter; yeast extract 0.5 g/liter.

and phosphorus sources. Several fertilizer-grade chemicals were tried as nutrient replacements with varying degrees of success (Table 11).

From these tests media were developed that used some of the fertilizer-grade and some of the laboratory-grade chemicals. A nitrogen-to-phosphorus-to-potassium (N-P-K) ratio of about 5 to 1 to 0.1 was maintained. These media were used in pilot-plant runs 6 through 15 (Table 12).

Fermenter and Feed Stream Sterilization

Before the start of each run the fermenter was filled with media and sterilized. All air and feed inlet and outlet lines, sampling lines and valves, inoculating ports, and acid and base inlet ports were sterilized at

TABLE 8

EFFECT OF PHOSPHATE LEVEL ON THE GROWTH OF CELLULOMONAS

Phosphate level (% phosphate)	N/P	Optical density (Klett units)	Cell yield[a] (dry wt) (g/liter)
0	-	40	0.052
0.004	15.7	100	0.13
0.01	6.3	200	0.26
0.02	3.1	220	0.286
0.04	1.6	265	0.345
0.08	0.8	265	0.345
0.2	0.31	200	0.26

[a] Cellulomonas grown in basal media plus various levels of phosphate for 48 hr in shake tube at 30 C.

the same time. Steam at 40 psig was used for sterilization of all lines, valves, and filters and was used in the fermenter jacket to heat the culture media. Initial sterilization temperature curves for the fermenter and all lines, filters, and ports show the severity of initial sterilization techniques (Figure 31).

When the cell density in the fermenter had reached the value desired, the continuous-feed sterilization section was sterilized and continuous-feed sterilization started.

The main feed pump and the stream injector recycle pump were started, and steam was fed into the injector. During this time, the pumped liquid stream was flowing through the sterilizing holding section, the evaporative

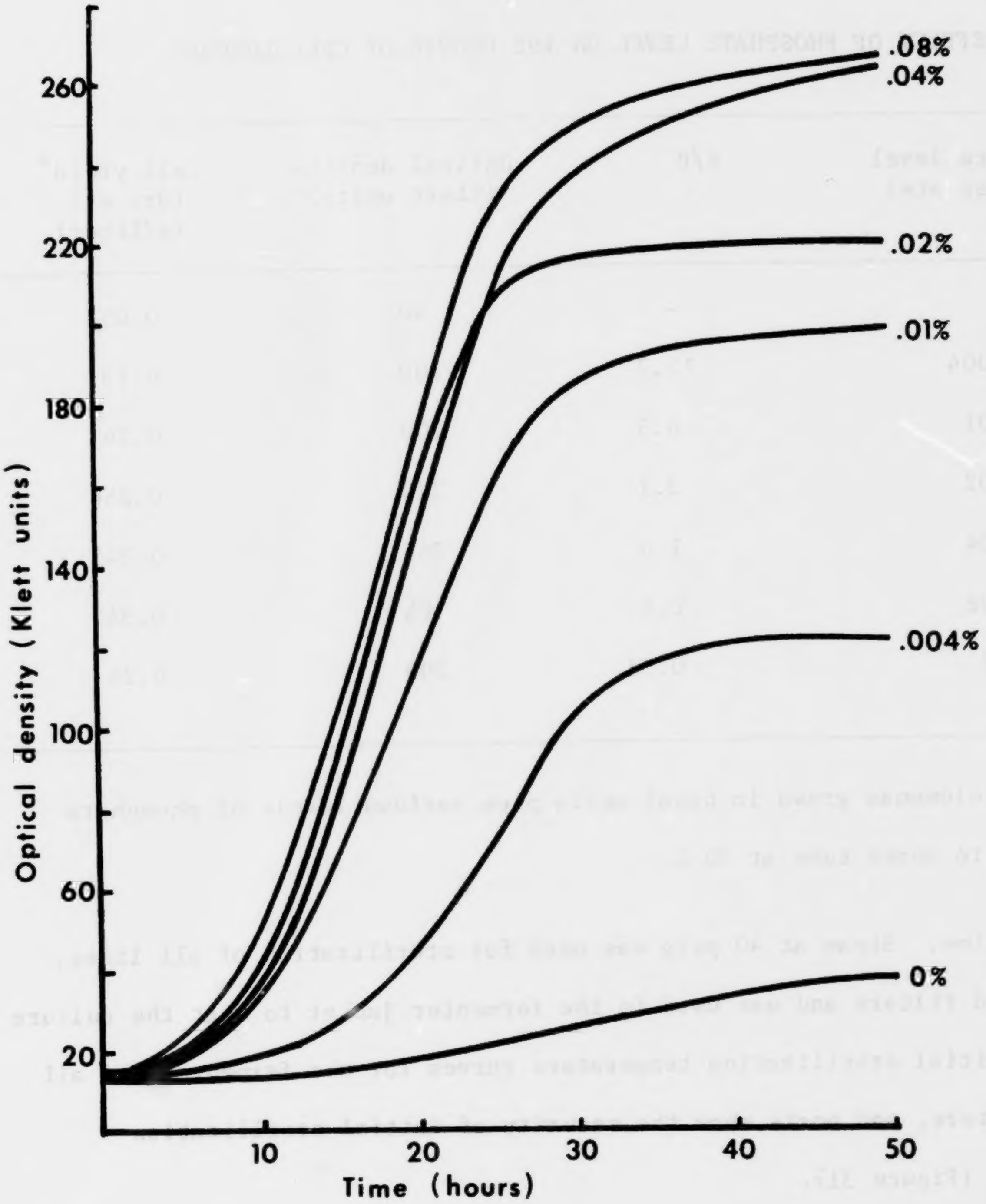


Figure 28. Effect of phosphate level on the growth of Cellulomonas.

TABLE 9

EFFECT OF SODIUM CHLORIDE LEVEL ON THE GROWTH OF CELLULOMONAS

NaCl level (% NaCl)	Klett unit	Cell yield[a] (dry wt) (g/liter)
0	125	0.104
0.1	135	0.175
0.3	145	0.190
0.6	100	0.130
1.0	60	0.078

[a] Cellulomonas grown in basal media for 24 hr on shake tube at 30 C.

cooler, and the chilled-water heat exchanger and was recycled to the reslurry tank or bulk feed tank.

The steam injector temperature control valve was set to maintain a pre-chosen temperature (usually 300 F) for liquid exiting the injector. This hot stream was flowed through the rest of the sterilizer system and recycled to the reslurry or feed tank. The sterilizing holding section, evaporative cooler, and chilled-water heat exchanger were heated to about 250 F for from 30 min to 1 hr before the coolers were started. After the heat exchangers and feed piping were sterilized, the cooling water was turned on in the evaporative cooler and the chilled-water heat exchanger. The temperature of the feed stream was then adjusted and allowed to reach equilibrium in all parts of the sterilizing-cooling section. Equilibrium temperatures were maintained in all parts of the continuous-feed sterilization section during a continuous fermentation (Figure 32).

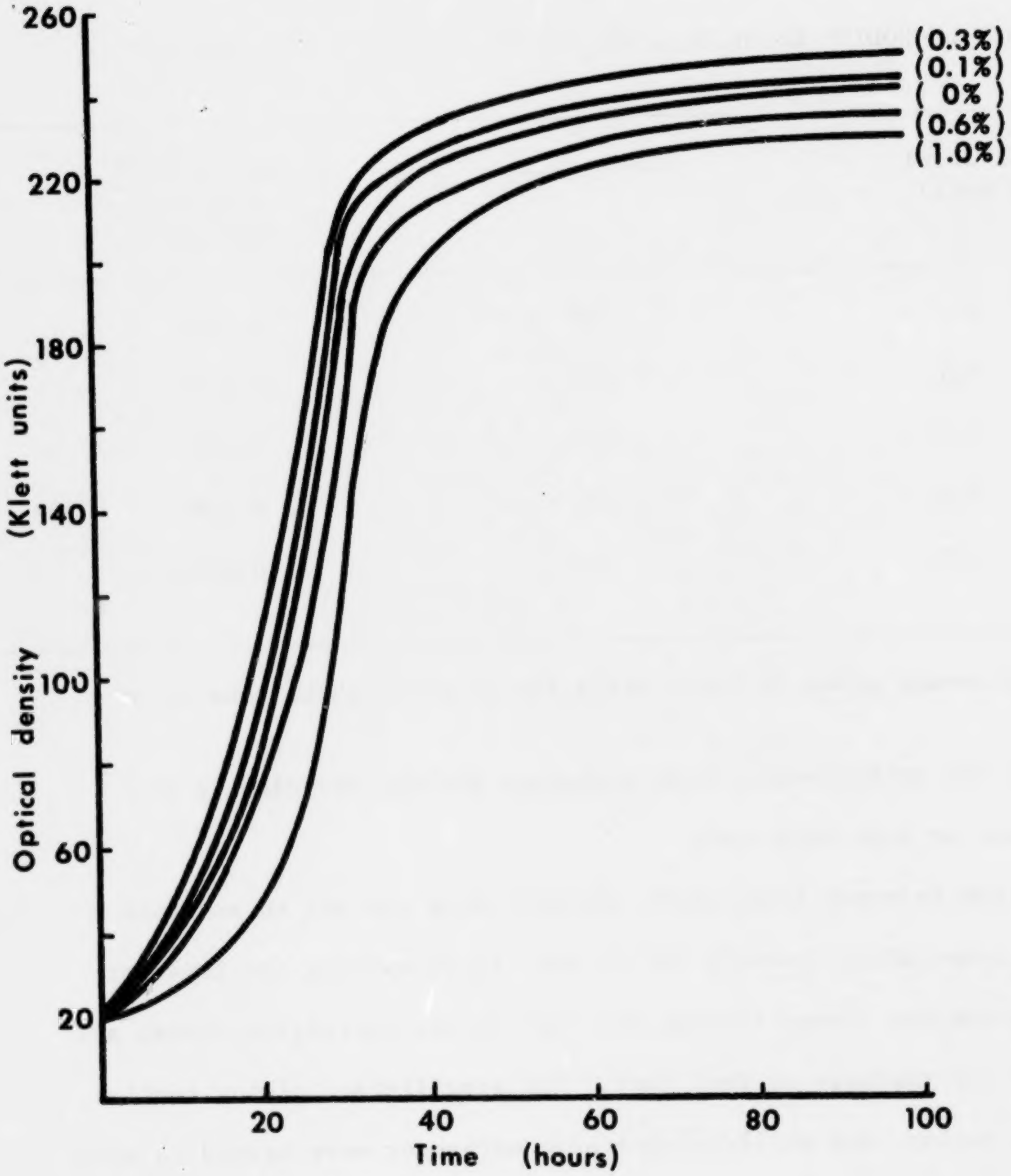


Figure 29. Effect of sodium chloride level on the growth of Cellulomonas.

TABLE 10

EFFECT OF TRACE MINERAL[a] LEVEL ON THE GROWTH OF CELLULOMONAS

Trace mineral[a] solution (ml/liter)	Klett unit	Cell yield[b] (dry wt) (g/liter)
0	216	0.280
0.1	219	0.285
0.5	228	0.297
1.0	230	0.300
5.0	210	0.274
10.0	26	0.034

[a] Mineral solution contains (g/liter):

$CaCl_2$	0.5
$FeCl_3 \cdot 6H_2O$	0.167
$ZnSO_4 \cdot 7H_2O$	0.18
$CuSO_4 \cdot 5H_2O$	0.16
$CoCl_2 \cdot 6H_2O$	0.18
EDTA	20.1

[b] Cellulomonas grown on basal media for 26 hr on rotary shaker.

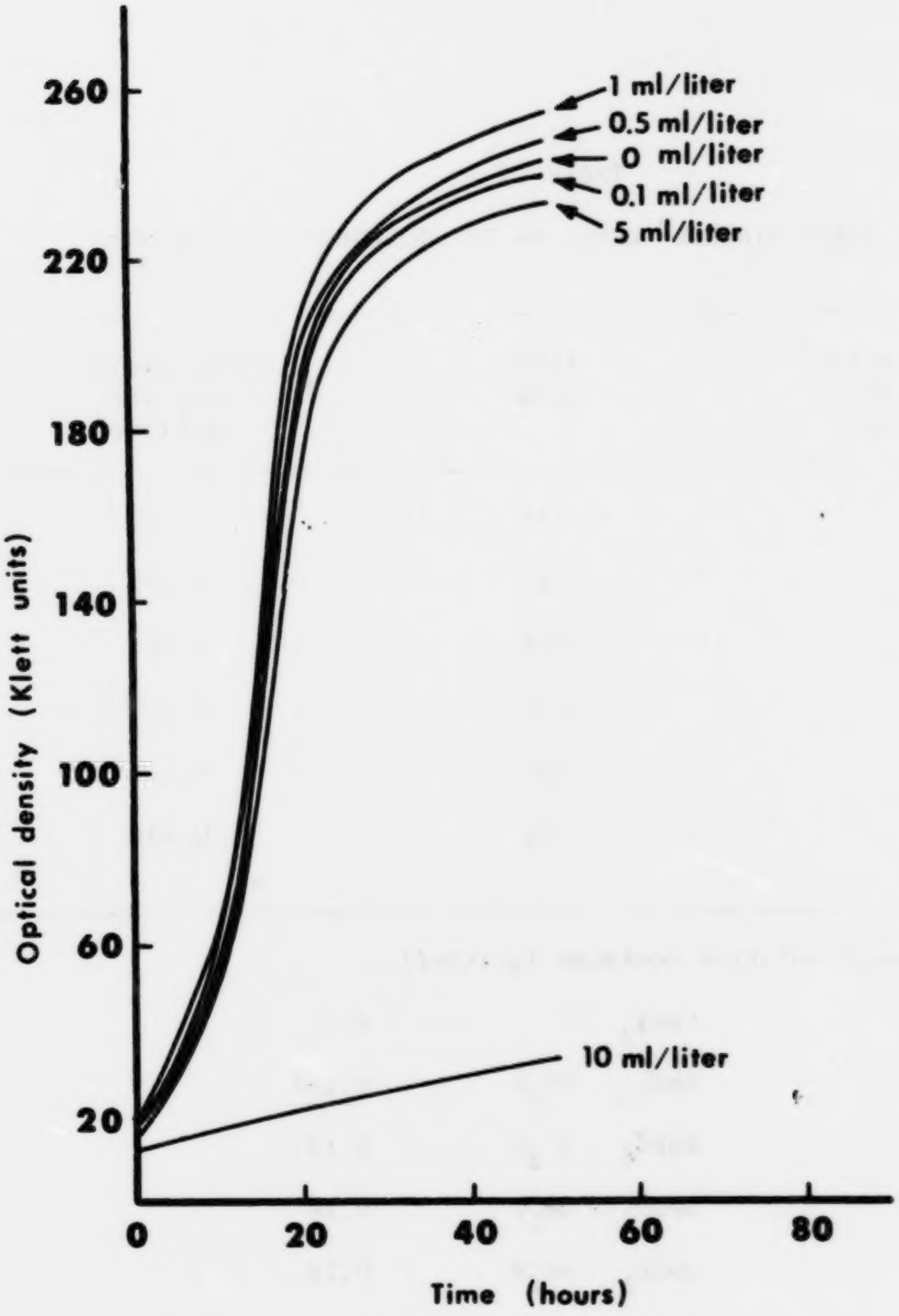


Figure 30. Effect of trace mineral level on the growth of Cellulomonas.

TABLE 11

REPLACEMENT INORGANIC NUTRIENTS

Nitrogen sources	Cell density Heavy	 Medium	 Light
Ammonium sulfate $(NH_4)_2SO_4$-as used in laboratory medium	X		
Urea, industrial grade (Company A)		X	
Urea, fertilizer grade (Company A)			X
Ammonium polyphosphate, TVA fertilizer grade	X		
Ammonium chloride, industrial grade	X		
Ammonium nitrate-urea, fertilizer mix (Company B)			X
Ammonium polyphosphate, fertilizer grade (Company B)			X

Work by Han and others has shown that the germicidal effect of sodium hydroxide considerably enhances the kill rate of bacteria and spores in a heat sterilization.[36]

For this experiment, a spore-forming bacterium was isolated from sugarcane bagasse. The spore suspension was then subjected to various combinations of time, temperature, and alkali concentration, and the rates of destruction were determined for each set of combinations. A series of survival curves and thermal-death time curves revealed a different mode of death between death by heat and by alkali. When alkali was incorporated with heat, the death rates of bacterial spores were increased and the slope of the thermal-death time curve was changed.

TABLE 12

PILOT PLANT NUTRIENT MEDIA FOR RUNS 6 THROUGH 15

Component	Amount (g per liter)
Substrate: treated bagasse or purified ground wood pulp	5.0
Ammonium polyphosphate (15 to 27.1 to 0)	0.73
Ammonium chloride, industrial grade	3.4
Sodium chloride, industrial grade	3.0
Calcium sulfate (or calcium chloride)	0.1
Magnesium sulfate	0.1
Potassium phosphate, dibasic	0.75
Yeast extract or yeast lysate[a]	0.2
Trace minerals solution, as in Table 5	1.0 ml
Polyglycol, P-2000	0.1 ml
Water	to 1.0 liter

[a] Yeast lysate was prepared by the concentration and lysing of brewers yeast obtained fresh from the Jackson Brewing Company, New Orleans, La.

From a series of thermal-destruction curves and alkaline-destruction curves, an empirical equation expressing the relationship between the death rate, temperature, and alkali concentration was established. The equation expresses that the death rate of the bacterial spore is affected exponentially by temperature and directly by alkali concentration. With the equation, sterilization time for various combinations of heat and alkali was determined, and the overall correlation index between the experimental data and the computed value was 0.877.

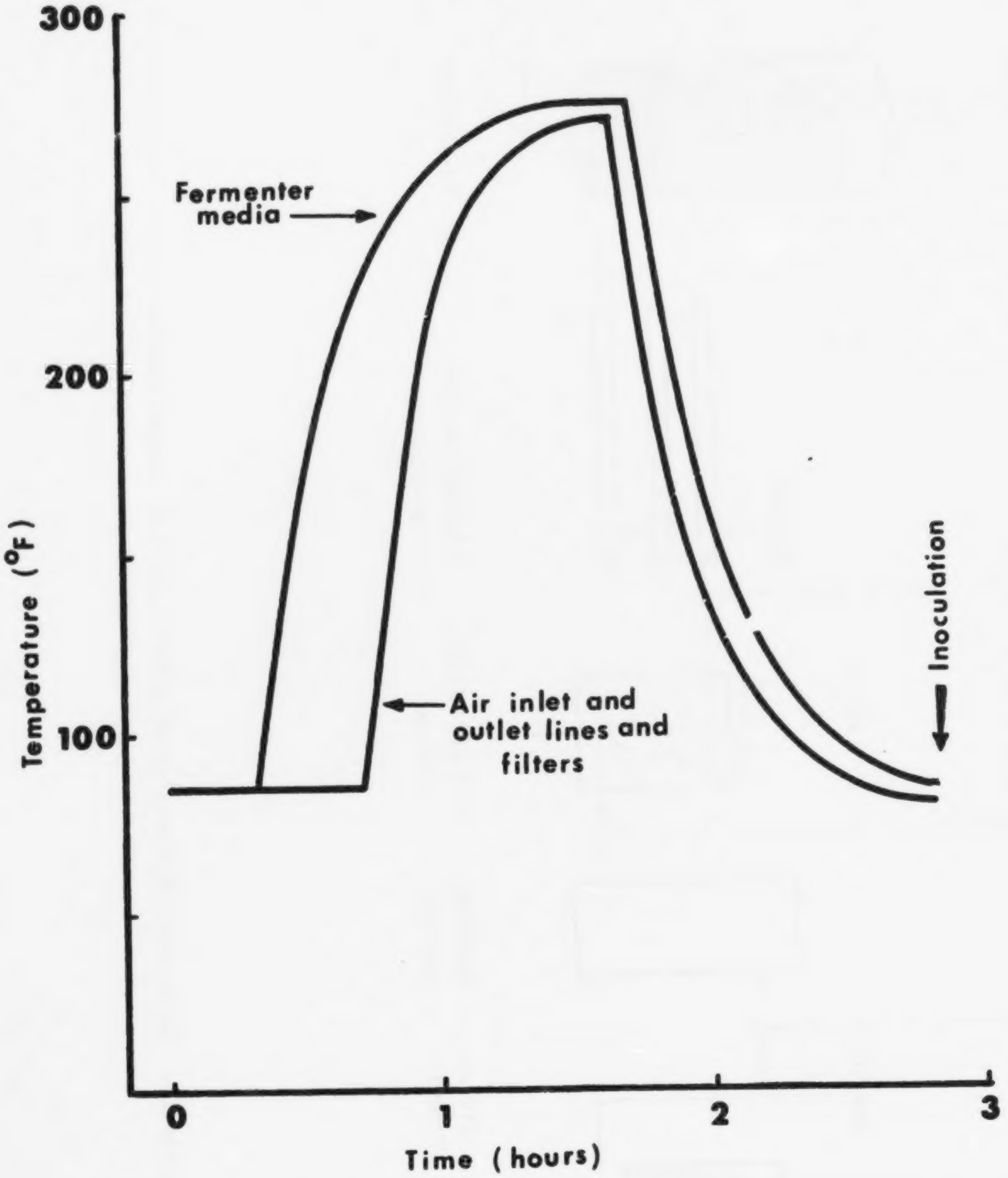


Figure 31. Initial sterilization profile.

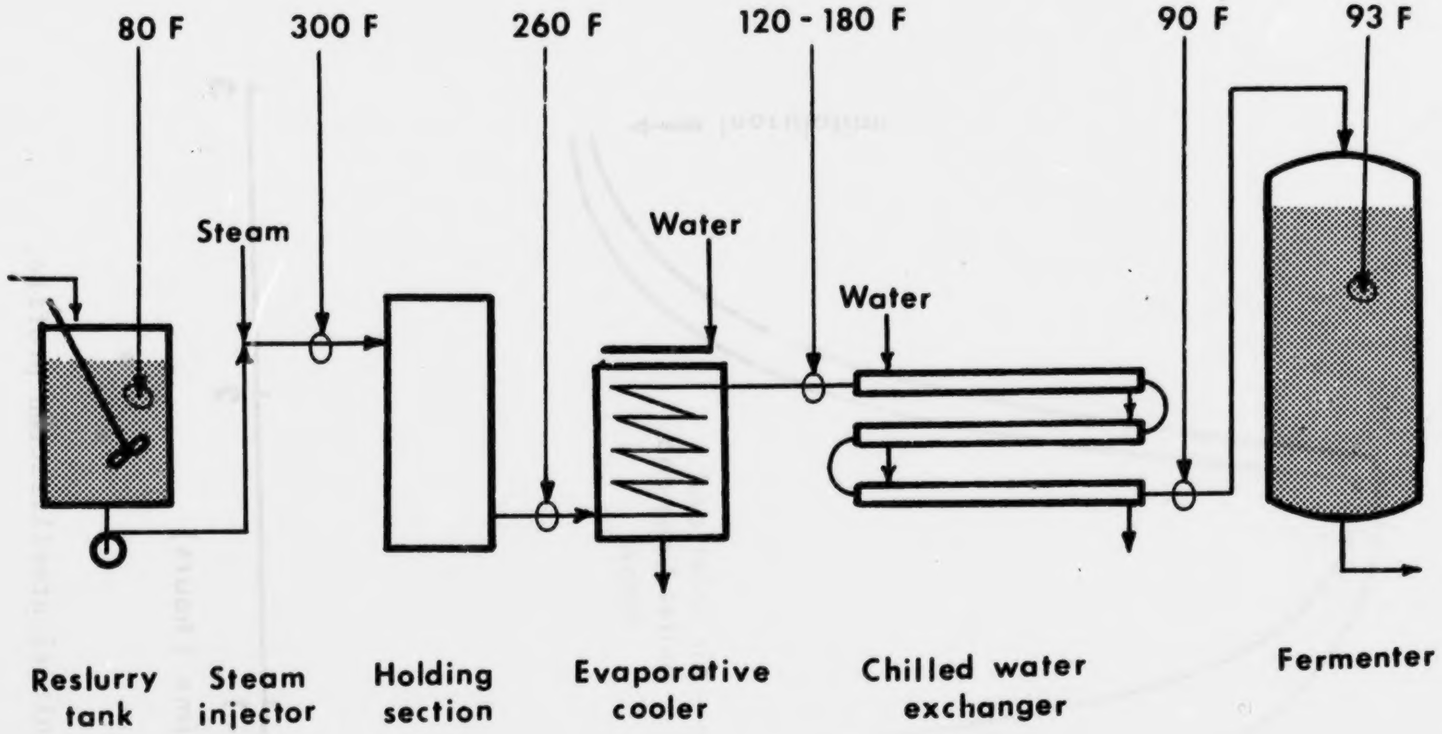

80 F
300 F
260 F
120 - 180 F
90 F
93 F
Steam
Water
Water
Reslurry tank
Steam injector
Holding section
Evaporative cooler
Chilled water exchanger
Fermenter

Figure 32. Equilibrium continuous-sterilization temperatures.

Inoculation and Fermentation

Cellulomonas, sp. bacteria were kept in pure slant cultures on nutrient agar. From the test tube containing a slant culture of Cellulomonas, a loopful of culture was transferred to a test tube containing sterile media (basal media plus a strip of filter paper) and incubated for 2 days until visual turbidity was observed. The actively growing culture was then propagated up to 15 liters by using 5 to 10 percent inoculum volume for each transfer step. Filter paper was used as a sole carbon source in early stages of propagation, and alakali-treated bagasse or ground wood pulp replaced filter paper in the 15-liter culture. All the flask cultures were incubated on a rotary shaker at 30 C, while the 15-liter carboy was incubated at room temperature and aerated with filtered air. The culture was allowed to reach a cell density of from 300 to 500 Klett units in the 15-liter carboy.*

When the temperature of the fermenter had cooled to the proper value (shown in Figure 31), the 15-liter inoculum was pumped in through the previously sterilized inoculation port. All fermentation variables were set at their proper control points, and batch growth of the organism was started.

The culture was agitated either by use of a draft tube or by a turbine agitator and baffle system. The draft tube left about 30 percent dead volume in the fermenter and was not as efficient in promoting oxygen transfer and mixing as the mechanical agitator was.[37] The mechanical agitator had essentially zero percent dead volume, promoted better oxygen transfer, and kept the contents of the vessel more homogeneously mixed. The agitator was usually run at 117 rpm, but subsequent runs have shown that higher agitation speeds promote faster growth rates and better oxygen transfer, and agitation speeds up to about 300 rpm have been used (Figure 33). The faster agitation rates, however, promote formation of a stable foam that is hard to break by addition of antifoam agent.

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*A Klett unit is a reading of optical density in a Klett-Summerson colorimeter; with Cellulomonas organisms, 1,000 Klett units equal a bacterial-cell density of about 1.3 g per liter dry weight.

Air used in fermenter aeration was taken directly from the 90 psi missile-grade air system of the building. The air was filtered before entering the fermenter in one of two parallel, steam-jacketed, glass fiber depth filters. Aeration rate was set and automatically controlled by the fermenter pressure-aeration rate control system. Aeration rate was usually set at about 2 volumes of air at standard temperature and pressure per volume of culture media per minute. This high rate was chosen to ensure a sufficient oxygen supply to the culture.

The concentration of dissolved oxygen in a growing culture was monitored in both draft tube-agitated and turbine-agitated cultures, and in pure Cellulomonas and mixed cultures. The transfer of oxygen from gas to liquid phase was directly effected by degree of agitation, and an increase in agitator speed was usually followed by an increase in growth rate (Figure 33). At high cell densities and high growth rates the culture would be limited by oxygen transfer if enough substrate was present. As substrate was consumed, growth would become substrate limited, and dissolved-oxygen concentration would gradually build up to saturation level.

The pH of the culture media was maintained between 6.5 and 6.7 during a run by addition of either acid or base. During active fermentation the pH tended to drop owing to the fermentative production of by-product and nucleic acids. During the growth phase only base, anhydrous ammonia, was

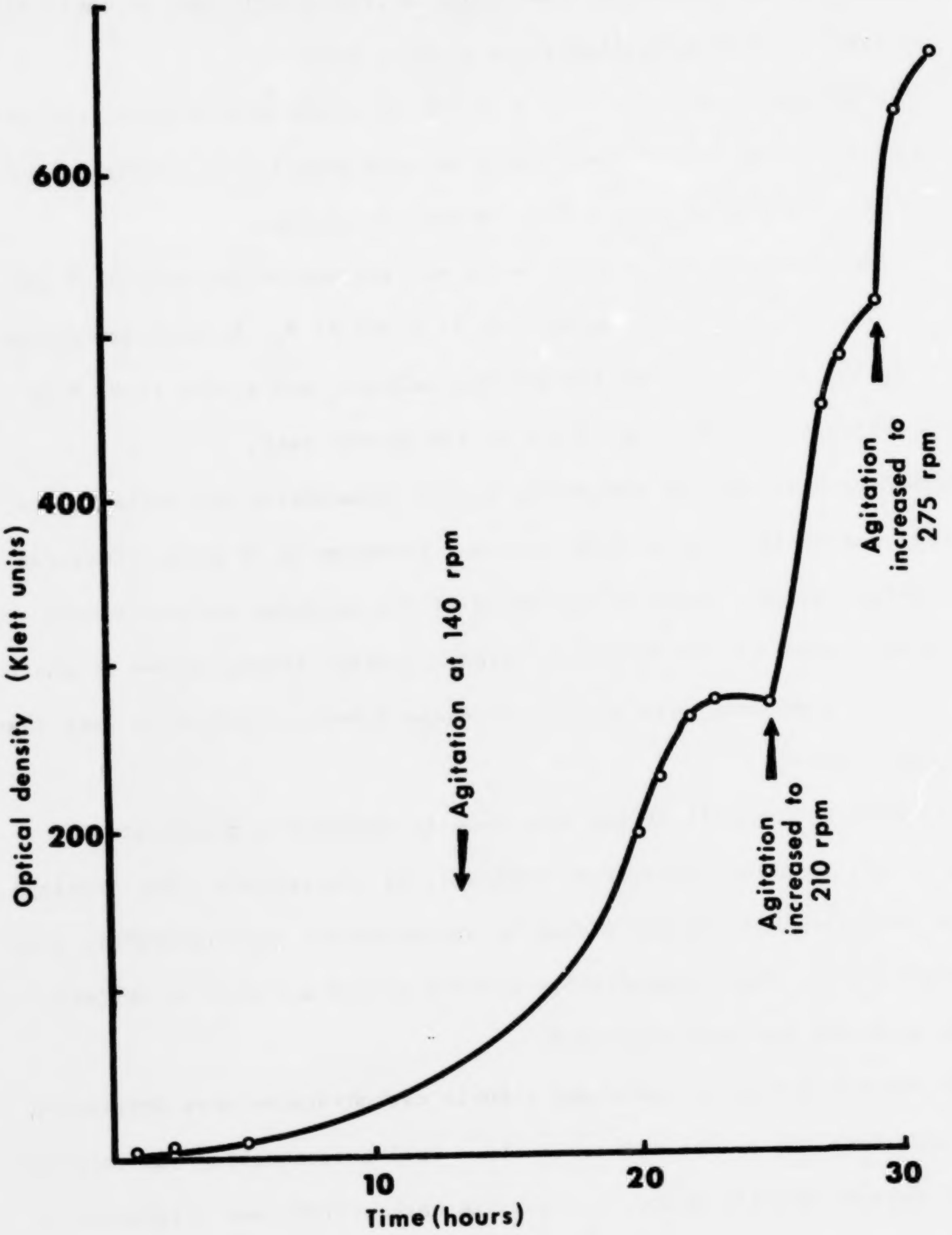

Figure 33. Effect of changing agitation in a batch fermentation.

added if the feed material was not too highly alkaline. If the process was left untended for any length of time during active growth, the pH would fall to 6.0 or lower, and fermentation would slow or cease.

If the pH rose to a value of 8.5 or 9.0 by addition of alkali, the acid produced by the fermentation would bring it back down to the proper range, but cellulase activity at such a high pH was not optimum.

The temperature of the culture media was automatically controlled and recorded. The usual setting was between 91 F and 94 F. A temperature rise to 104 F or 105 F did not hurt the growing culture, and a drop to 80 F or 85 F had only a slight slowing effect on the growth rate.

Fermenter pressure was controlled by the fermenter's air outlet valve. The vessel was usually run with an internal pressure of 20 psig. This was done to permit smooth, positive operation of the aeration control system and to minimize chances of contamination through leakage around valves or shaft seals. Higher pressures were used to increase dissolved oxygen in runs that were oxygen limited.

Note here that no effort has been made to define the quantitative effects of pH, aeration, agitation, pressure, or temperature. The settings of these variables were chosen either by extrapolation from laboratory data or by experience. Their quantitative effects should and will be defined, but that work has not been completed.

The concentrations of cells and soluble carbohydrates were determined by periodic sampling of the culture media. Cell concentration was reported in Klett optical density units, and soluble carbohydrate was determined as mg per liter. Cell and soluble-carbohydrate concentrations for a batch fermentation where unwashed alkali-oxidation-treated sugarcane bagasse was used as substrate are presented (Figure 34). The cell density curve for a continuous run is also presented (Figure 35). Typical curves are presented

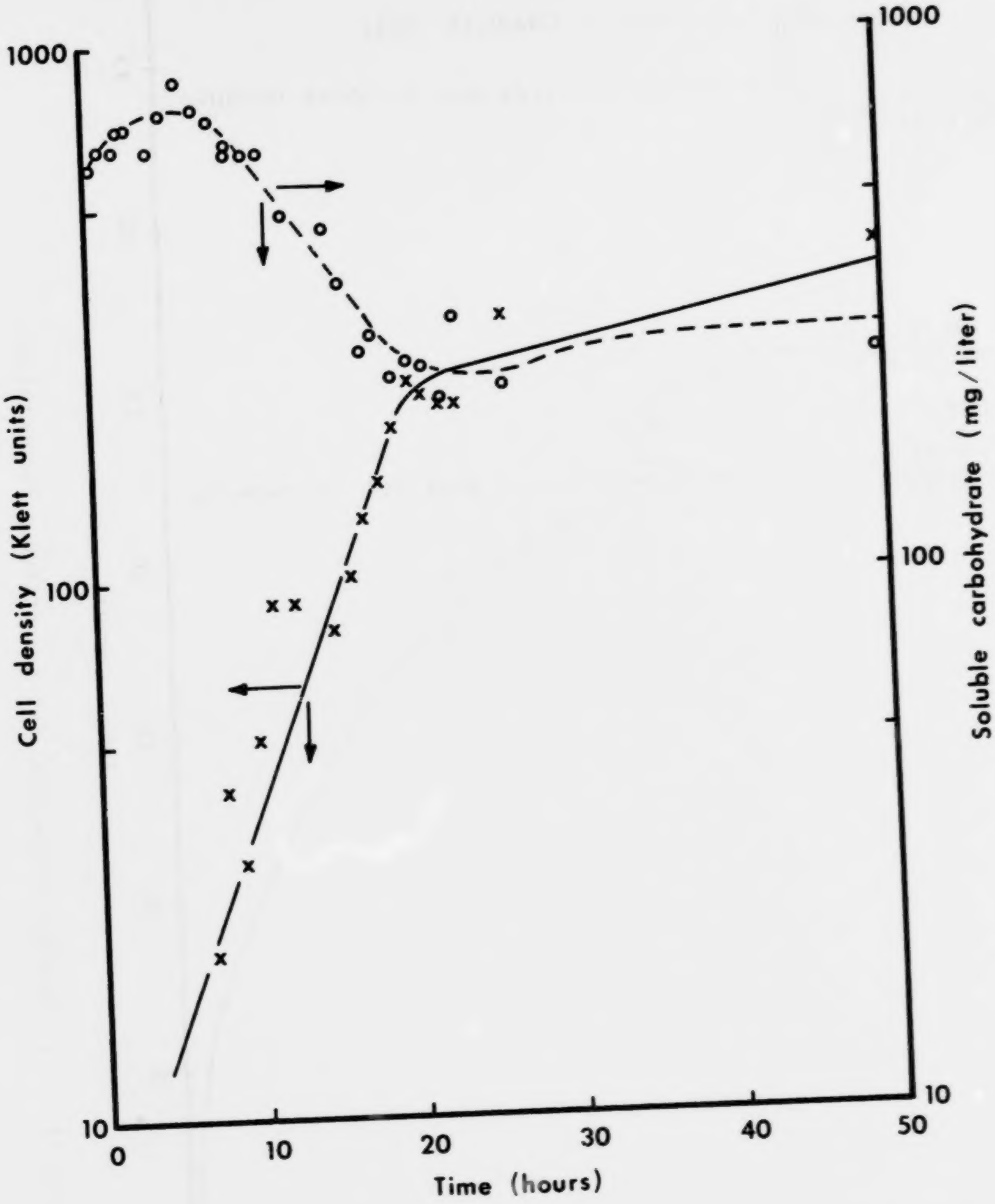

Figure 34. Cell density and soluble carbohydrate concentration versus time for a batch fermentation.

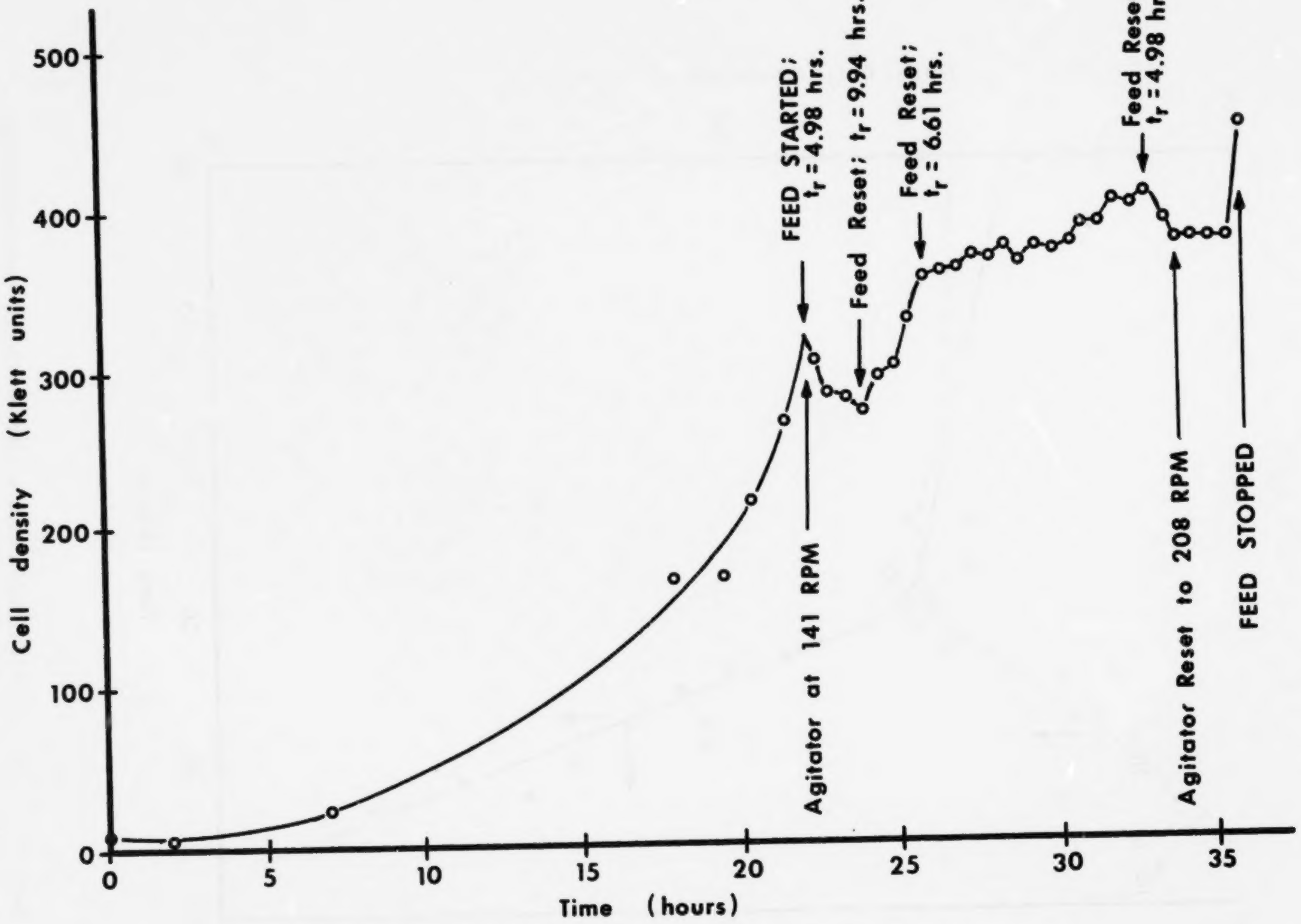

Figure 35. Cell density versus time for a continuous fermentation.

for the same variables in batch runs with unwashed, treated bagasse (Figure 36) and washed, treated bagasse or wood pulp (Figure 37).

Values for cell density versus time have been collected for all pilot-plant batch and continuous runs. If the data from the batch runs are analyzed by the method of Adams and Hungate, constants may be evaluated for use in the continuous-flow process.[38]

For evaluating the cell production potential for a particular batch culture in a continuous-flow fermenter, the cell concentration (X) is plotted versus the time (t) (Figure 38). Then the slope of the growth curve dX/dt is taken at several points and plotted versus the cell concentration (Figure 39). For finding the growth rate constant (k), the slope of a line from the origin to any point on the dX/dt-versus-X curve is taken. In a single stage, backmix fermenter k equals D (the dilution rate, hours^{-1}) if the cell growth is substrate limited. The predicted equilibrium cell concentration may be taken from the dX/dt-versus-X curve by dropping perpendicularly to the ordinate X and reading the concentration. A curve of all cell production (P) may then be plotted, which is the feed rate times equilibrium cell concentration versus the feed rate, to find the optimum operating range of the continuous fermenter (Figure 40). The maximum volumetric production efficiency (VPE) of the fermenter may be calculated as grams dry cells per liter per hour.

In continuous runs, the feed stream was started during the log phase of growth (Figure 35). An arbitrary feed rate was chosen initially, and the cell density usually rose or fell depending on the rate of feed. The feed rate was adjusted to the desired value to permit the cell density to reach equilibrium. Volumetric production efficiency was then determined and some variable changed. The cell density was again allowed to reach equilibrium

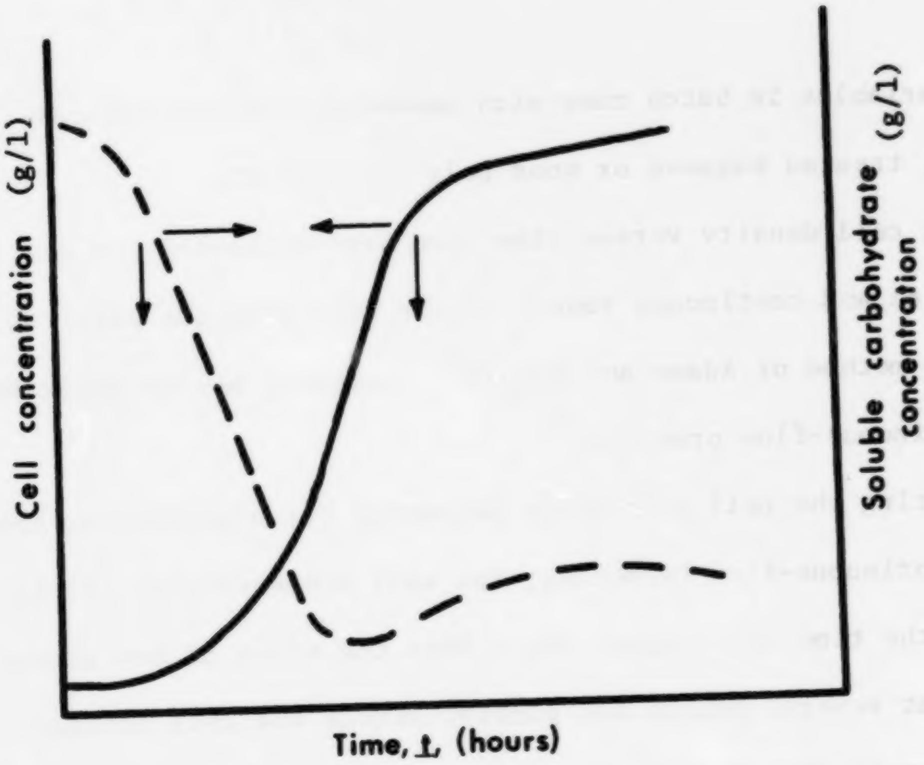

Figure 36. Fermentation of unwashed, treated bagasse.

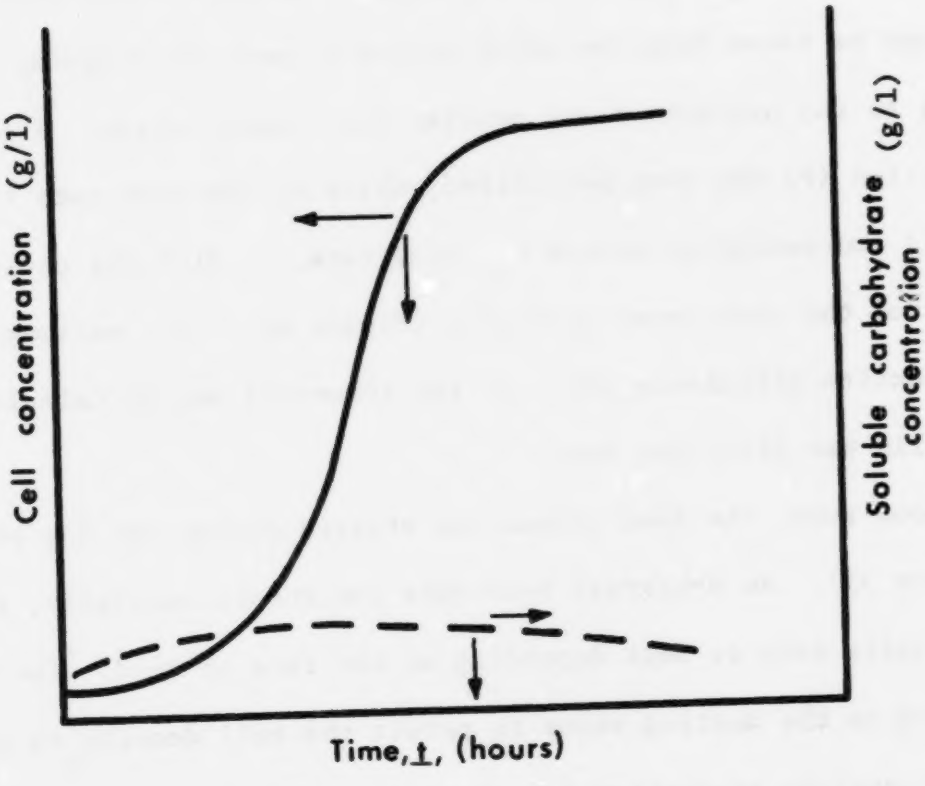

Figure 37. Fermentation of washed, treated bagasse or purified wood pulp.

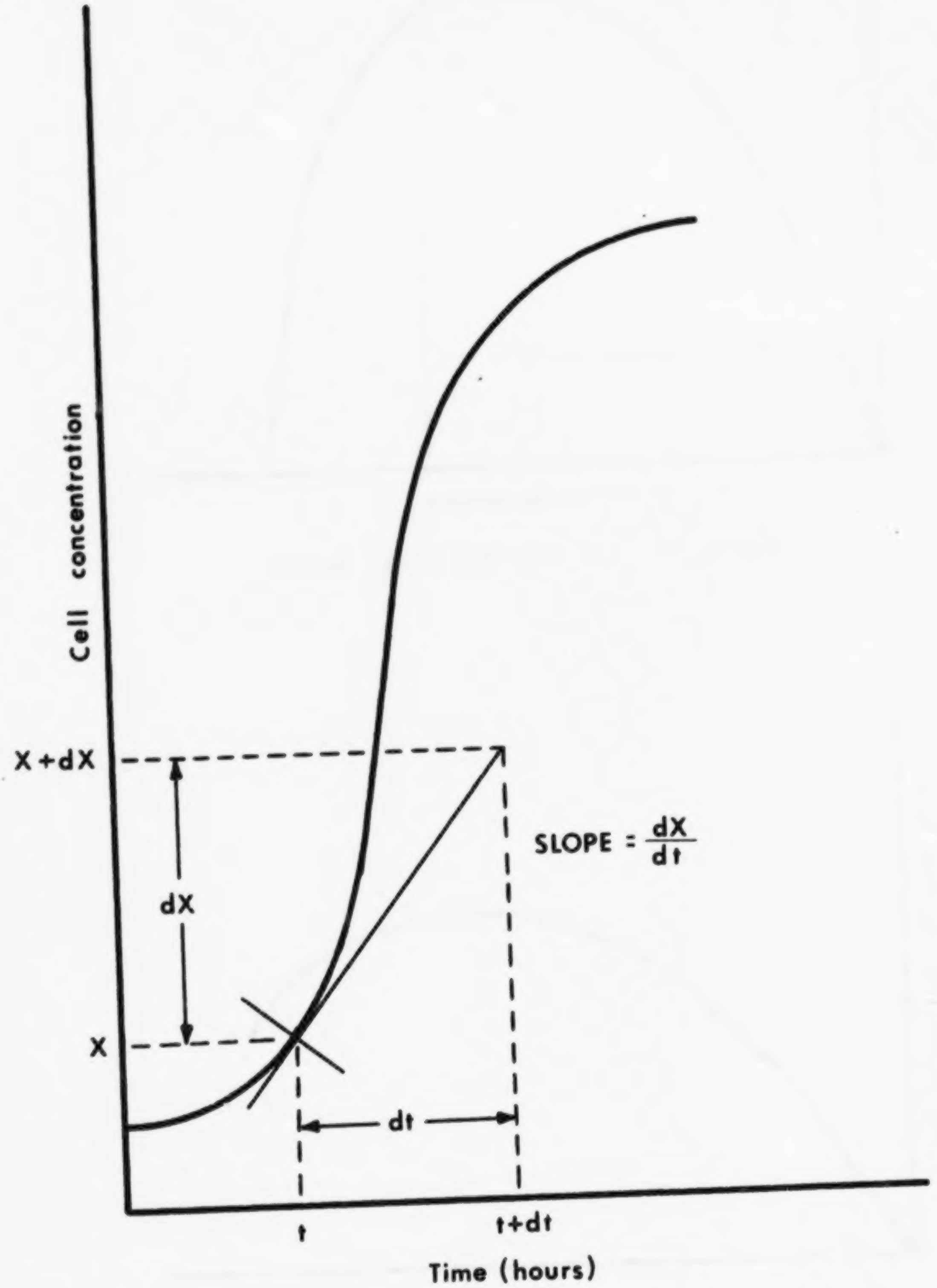
Cell concentration
X+dX
dX
X
SLOPE = $\frac{dX}{dt}$
dt
t
t+dt
Time (hours)

Figure 38. Calculation of $\frac{dX}{dt}$ values.

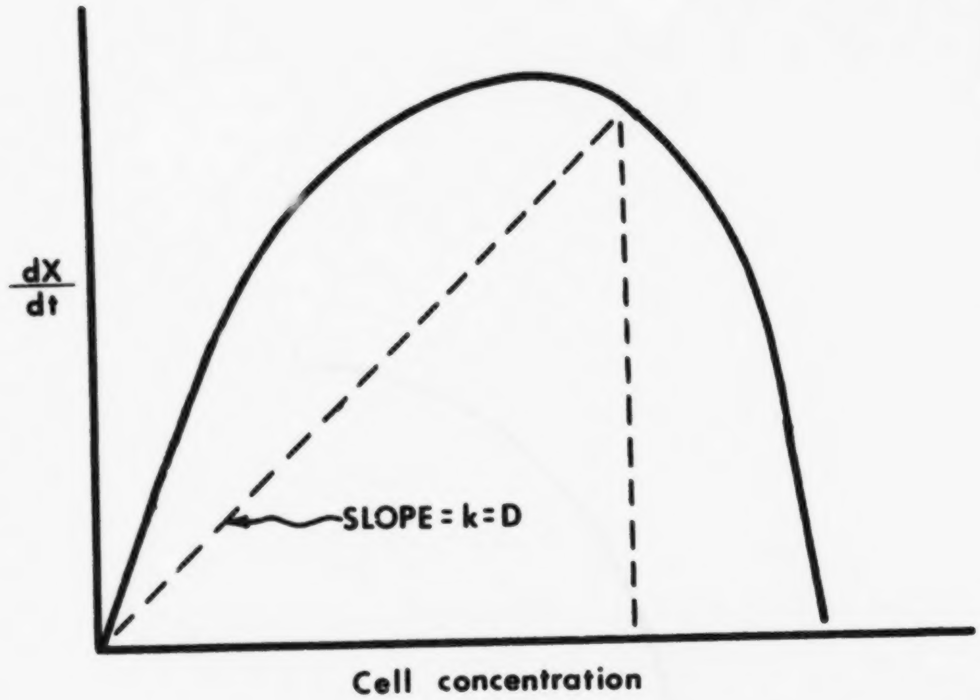

Figure 39. Calculation of k values.

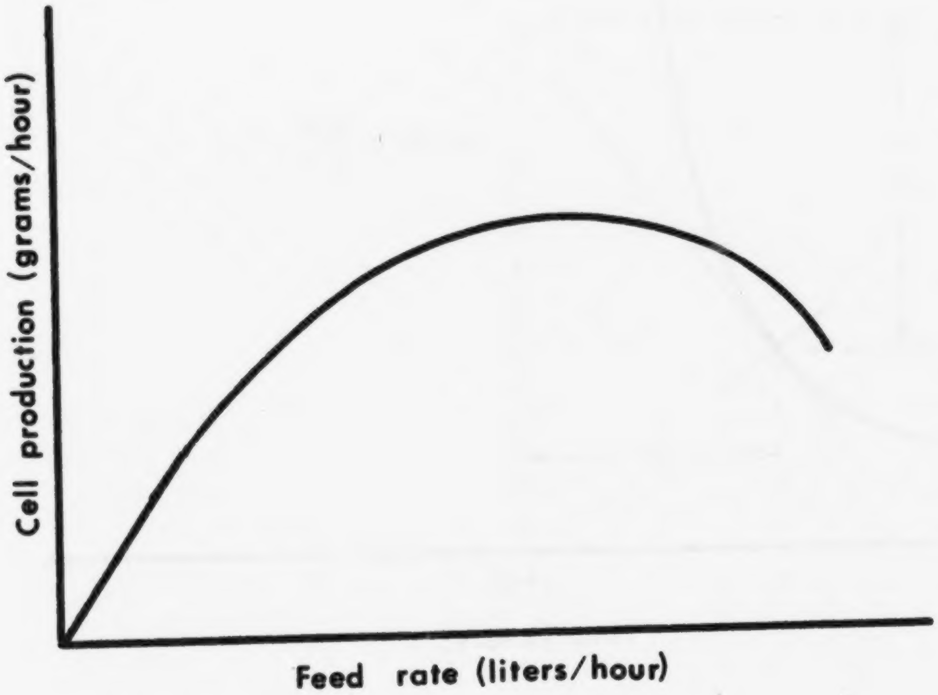

Figure 40. Determination of maximum cell production and optimum feed rate.

and the VPE again calculated. This procedure was continued to the end of the run.

Data from all successful batch and continuous runs of the pilot plant were collected and used for calculation of fermentation rates and efficiencies (Table 13). Batch runs of from 24-hr to 182-hr duration were made, and continuous flow was maintained for from 30 to 74 hr. Bagasse or wood pulp was initially charged at from 5 to 10 g per liter of media, and the continuous-feed streams held 5 g per liter. From 53 to 91 percent of the bagasse fed was solubilized during the batch runs, the longer runs generally consuming more of the bagasse. This was not strictly true in all cases, and it is felt that the initial alkaline-oxidation treatment may control the initial degree of bagasse solubilization. The more severely treated bagasse dissolves (and is thus metabolized) more thoroughly than bagasse with more mild treatment. Continuous-flow cultures could probably be maintained at a utilization efficiency of from 50 to 70 percent, depending on residence time.

Log-phase cell mass-doubling times ranged from 1.8 to 4.5 hr for bagasse, and from 2.5 to 5 hr for purified wood pulp. Average cell mass-doubling time for bagasse-grown cultures was about 3.6 hr. VPE values were calculated from all batch-run data, and the values ranged from 0.02 to 0.162 for pure cultures and 0.512 for the symbiotic culture. Experimental continuous VPE values were 0.033 and 0.098. This showed that about 1 g of dry SCP could be produced for each 6 liters of pure Cellulomonas culture volume every hour.

When unwashed, alkali-oxidation-treated bagasse was used as the substrate in a run, the initial level of soluble carbohydrate was from 500 to 800 mg/liter. The level depended on the severity of the pre-fermentation

TABLE 13

FERMENTATION BATCH AND CONTINUOUS RUN DATA FOR 141-gal PILOT-PLANT FERMENTER

	Run Number			
	4B[a]	4C[a]	5	6
Length of run (hr)	30	74	27	24
Weight of initial bagasse substrate (g dry weight)[d]	3120	5.0	3200	2650
Weight of bagasse recovered after fermentation (g dry weight)	--	--	1500	633
Percent bagasse solubilized	--	--	53.0	76.0
Weight of cells at end of batch (g dry weight)	244	--	340	259
Log phase mass doubling time, (t_{md}-hr)	4.4	--	1.8	3.7
Log-phase growth-rate constant[f] (hr^{-1})	0.145	--	0.24	0.189
Maximum cell density (g dry weight/liter)	0.47	0.17	0.64	0.49
Calculated volumetric production efficiency[b]	0.0525	--	0.070	0.0675
Actual volumetric production efficiency (maximum)[c]	--	0.033	--	--

[a] B and C designations represent the respective batch and continuous flow parts of the same run. Values given are for the culture in equilibrium.

[b] Volumetric production efficiency is given as g of dry cell mass produced per liter of culture media per hr. These values are calculated from batch data.

[c] Volumetric production efficiency values experimentally determined from continuous run data.

TABLE 13 (Continued)

FERMENTATION BATCH AND CONTINUOUS RUN DATA
FOR 141-gal PILOT-PLANT FERMENTER

	Run Number			
	7	8	9	10
Length of run (hr)	117	90	182	CONTAMINATED
Weight of initial bagasse substrate (g dry weight)[d]	2650	2650	2630	
Weight of bagasse recovered after fermentation (g dry weight)	299	248	--	
Percent bagasse solubilized	89.0	90.8	--	
Weight of cells at end of batch (g dry weight)	434	890	417	
Log-phase mass-doubling time, (t_{md}-hr)	3.2	3.3	3.9	
Log-phase growth-rate constant[f] (hr^{-1})	0.128	0.140	--	
Maximum cell density (g dry weight/liter)	0.82	1.66	0.78	
Calculated volumetric production efficiency[b]	0.033	0.056	--	
Actual volumetric production efficiency (maximum)[c]	--	--	--	

[d] Cellulosic weights are given as g initially charged in batch runs and as grams per liter of feed in the continuous runs.

[e] Purified ground wood pulp.

[f] Log-phase growth rate at maximum theoretical volumetric production efficiency.

TABLE 13 (Continued)

FERMENTATION BATCH AND CONTINUOUS RUN DATA FOR 141-gal PILOT-PLANT FERMENTER

	Run Number			
	11	12	13	14B[a]
Length of run (hr)	48	114	46	21.5
Weight of initial bagasse substrate (g dry weight)[d]	2650	2650[e]	2650	2650
Weight of bagasse recovered after fermentation (g dry weight)	--	--	--	--
Percent bagasse solubilized	--	--	--	--
Weight of cells at end of batch (g dry weight)	410	446	710	539
Log-phase mass-doubling time, (t_{md}-hr)	3.0	5.0	4.0	3.6
Log-phase growth-rate constant[f] (hr^{-1})	0.114	0.075	0.0769	0.1768
Maximum cell density (g dry weight/liter)	0.77	0.96	1.33	1.005
Calculated volumetric production efficiency[b]	0.055	0.0197	0.056	0.069
Actual volumetric production efficiency (maximum)[c]	--	--	--	--

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[g] Symbiotic run with *Alcaligenes faecalis* and Cellulomonas.

TABLE 13 (Continued)

FERMENTATION BATCH AND CONTINUOUS RUN DATA
FOR 141-gal PILOT-PLANT FERMENTER

	Run Number			
	14C[a]	15	16	17[g]
Length of run (hr)	28.5	CONTAMINATED	32	69.5
Weight of initial bagasse substrate (g dry weight)[d]	5.0		5.0[e]	10.0
Weight of bagasse recovered after fermentation (g dry weight)	--		--	--
Percent bagasse solubilized	--		--	--
Weight of cells at end of batch (g dry weight)	--		465	3340
Log-phase mass-doubling time, (t_{md}-hr)	--		3.9	4.5
Log-phase growth-rate constant[f] (hr^{-1})	--		0.20	0.0532
Maximum cell density (g dry weight/liter)	0.52		0.871	6.24
Calculated volumetric production efficiency[b]	--		0.162	0.512
Actual volumetric production efficiency (maximum)[c]	0.098		--	--

treatment and on the concentration of bagasse fed. When washed bagasse and wood pulp were used, the soluble-carbohydrate level was from 10 to 100 mg/liter.

Cellulomonas grew on all three substrates; however, substrate utilization mechanisms seemed to be quite different. The soluble-carbohydrate concentration of cultures with high inital values fell quite rapidly during lag and initial logarithmic phases (Figure 34). Both the cell mass increase and the soluble-carbohydrate decrease may be determined quantitatively during this time. When both these values were calculated over a discrete time period, as long as it was in the logarithmic phase of cell growth, it was seen that the yield coefficient--grams of cell mass increase per gram of soluble-carbohydrate decrease--was 0.5, or 50 percent. This is the accepted value for aerobic cell growth on a carbohydrate substrate. This was interpreted to mean that if metabolizable soluble carbohydrate is present in the media in sizable amounts, it will be metabolized before the insoluble cellulose. This has, of course, been well recognized previously and has been attributed to repression or inhibition of cellulase enzymes by the soluble carbohydrate, particularly cellobiose.

When the soluble carbohydrate reached a low level in these batches, the growth rate slowed considerably.

In batches where washed, treated bagasse was used, the initial concentration of soluble carbohydrate ranged from less than 10 to 100 mg/liter. The cells grew and exhibited almost the same kinetics as when grown with larger soluble-carbohydrate concentrations, and the concentration of soluble carbohydrate did decrease somewhat. The soluble carbohydrate no longer supplied, however, a sufficient quantity of substrate to explain cell mass increase. The insoluble cellulose was, therefore, being degraded and metabolized to some extent whenever initial soluble carbohydrates were low, and the transfer of insoluble cellulose to soluble carbohydrate by enzyme action was appreciable.

In batches with pure wood pulp as the substrate, the initial concentration of soluble carbohydrate was usually less than 50 mg/liter. During the run, the concentration increased until the end of the log-growth phase and then decreased (Figure 37). It never rose above about 150 mg/liter. In these cultures all the soluble substrate was being produced by enzymatic breakdown of cellulose.

Y. W. Han has found that the severity of the alkaline-oxidation treatment of bagasse directly affects the amount of carbohydrate that is solubilized but, after a certain point, does not seem to increase the rate or level of organism growth.[36] These data indicate that the usefulness of the thermal-oxidation step is minimal, and it is distinctly harmful if an active cellulase enzyme system is desired. In other words, it may be best to minimize oxidative breakdown of cellulose. A certain mild alkali swelling treatment is, however, necessary since untreated bagasse is metabolized at an extremely slow rate.

The rate of mechanical agitation was shown to have a definite effect on the limit and rate of growth of the organism (Figure 33). In a batch run the agitation rate was changed twice after cell growth started. Each time the growth rate increased after an increase in agitation rate. After a period of growth, the culture would begin to enter the stationary phase. Another increase in agitation would initiate another cycle. It is presumed that the increased agitation led to higher dissolved-oxygen transfer rates and relieved the oxygen limitations of the culture.

Harvesting

The SCP product and the by-product undigested cellulose were harvested from both batch and continuous-flow cultures by one of three methods, depending on the type product desired.

The cellulose waste that passed through the fermenter without being solubilized had to be cleared from the product stream. This was done either by direct filtration of the effluent stream by a 40-mesh screen filter or by settling. All but the fine particles of fiber could be removed from the stream with the filter. An even cleaner stream could, however, be obtained by batch or continuous settling of the product stream. This was accomplished in a variable volume, and thus, a variable residence time mixer/settler. A residence time of from 2 to 6 hr was provided in this vessel, and a cleared overflow stream was obtained. The undigested cellulose could then be taken off as an underflow.

The overflow stream containing the cells was flowed either to the centrifuge or to the flocculent addition tank and the second mixer/settler unit. Cells were precipitated in the second mixer/settler and removed as an underflow in a heavy cream. Organisms spun out of the media by centrifugation were removed from the centrifuge as a heavy sludge.

The cell cream from the second mixer/settler could be drum dried or freeze dried directly from the settler. The underflow stream contained from 4 to 5 percent solids by weight. When this stream was dried without further cell concentration, however, a large concentration of salts was obtained in the product. The salts came from the nutrient inorganics that passed through the fermenter unchanged and from those that were generated in the fermenter. Salt concentrations in the harvested dry cell product have run as large as 25 percent.

The cell cream from the precipitation step could be centrifuged prior to drying to remove most of the slats. Centrifugation of the precipitated cream was a much easier and more economical step than that of the total cellulose-free media.

The dried SCP product contained from 50 to 60 percent crude protein as determined by Kjeldahl analysis, and was quite low in fiber and lignin (Table 14).

TABLE 14

PRODUCT ANALYSIS[39]

Sample	Selected component (% on a dry basis)					
	Protein	Fat	Fiber	Ash	Lignin	ADF[a]
Untreated bagasse	2.92	1.87	40.6	4.68	7.6	53.5
Treated bagasse	1.66	1.59	39.2	23.4	3.07	46.1
Freeze-dried, centrifuged cells	57.8	2.53	2.53	9.0	1.37	3.7
Cellulose remaining after fermentation	7.7	2.67	68.0	2.88	7.6	74.0

[a] ADF is acid detergent fiber.

The cellulose remaining undigested or insoluble after the fermentation contained a much larger relative fiber content than the unfermented bagasse. It is probable that the alkali-oxidation treatment and the enzyme action in the fermenter degraded and solubilized protein, fat, lignin, and hemicelluloses, leaving a relatively larger fraction of insoluble fiber in the recoverable effluent solids. This fiber was essentially de-pithed and clean and had a higher relative crystallinity than the unfermented samples.

PRODUCT QUALITY AND BY-PRODUCT USAGE

Cellulomonas sp bacteria were grown under controlled conditions in the laboratory on a carboxymethyl cellulose substrate. An analysis of the harvested cells showed a protein content of 46.2 percent and a nonproteinaceous nitrogen level of 7.7 percent on a dry-weight basis[40] (Table 15).

The essential amino acid content of the cell protein of the organism was determined, and the values obtained were compared with the FAO reference-protein values and with those of proteins from other plant and animal sources (Table 16). Also included are the analyses of single-cell proteins from petrochemicals. The essential amino acid pattern of the Cellulomonas compares favorably with that of FAO reference protein. The lysine content, which is deficient in a number of foods, particularly cereal grains, was larger than that of the reference protein. The content of other essential amino acids, such as leucine and valine, were extremely large when compared with the proteins of other sources and the FAO reference protein. The methionine content was comparable with that of wheat flour or single-cell protein produced from hydrocarbon.

Feeding studies were conducted on male weanling rats of the Sprague-Dawley strain.[41] It was found the Cellulomonas cells were superior to Pseudomonas cells produced on hydrocarbons but were inferior to casein. The rats held their weight on a diet with 20 percent protein supplied by Cellulomonas and showed gains on a diet containing 40 percent Cellulomonas protein. The cells were not toxic even when fed at the 80 percent level (40

TABLE 15

GROWTH YIELDS OF CELLULOMONAS ON CARBOXYMETHYL CELLULOSE

	Yields (mg/mg) in 100 ml	(%)
Cell mass[a]/CH_2O consumed[b]	13.0/26.0	50.0
Protein[c]/cell mass	6.0/13.0	46.2
Nonprotein-N[d]/cell mass	1.0/13.0	7.7
Protein/CH_2O consumed	6.0/26.0	23.0

[a] Cells grown 2 days on a basal medium containing 0.1% of CM-cellulose were harvested by centrifugation. Cell crops were dried at 110 C to obtain a constant weight.

[b] Difference of CH_2O concentrations in initial and final medium. CH_2O concentrations were measured by phenol sulfuric acid method.

[c] By micro-Kjeldahl method after extracting nucleic acids with 5 percent TCA at 90 C for 30 min.

[d] Difference of N content in whole cells and hot-TCA-treated cells.

percent crude protein). The addition of L-methionine improved the quality of the protein considerably. Thus, it was believed that methionine is the first limiting amino acid of Cellulomonas protein. Large fecal-nitrogen content of the rats fed intact Cellulomonas cells indicated the resistance of the cell wall of Cellulomonas to digestion. It was felt that cell homogenization or lysis before feeding would improve the efficiency of protein utilization.

The SCP product when dried and ground is a free-flowing powder with a dark brown-to-yellow color depending on amount of lignin inclusion (Figure 41). At low moisture levels the storage properties and shelf life are very

TABLE 16

ESSENTIAL AMINO ACID CONTENT OF THE CELL PROTEIN[32] (g OF AMINO ACID PER 100 g PROTEIN)

Amino acid	Cellulomonas[a] cell protein	FAO[b] reference protein	Wheat[c] flour	Beef[c]	BP[c] protein
Arginine	9.21	--	4.2	7.7	5.1
Histidine	2.30	--	2.2	3.3	5.1
Isoleucine	4.74	4.2	4.2	6.0	4.6
Leucine	11.20	4.8	7.0	8.0	3.1
Lysine	6.84	4.2	1.9	10.0	6.0
Methionine	1.86	2.2	1.5	3.2	1.1
Phenylalanine	4.36	2.8	5.5	5.0	8.1
Tyrosine	2.67	2.8	--	--	--
Threonine	5.37	2.8	2.7	5.0	11.0
Valine	10.71	4.2	4.1	5.5	7.0

[a] The sample was hydrolyzed with 6 N HCl at 100 C for 22 hr and analyzed with a Beckman model 116 amino acid analyzer in the laboratories of Dr. S. P. Yang, School of Home Economics, Louisiana State University.

[b] National Academy of Science - National Research Council.

[c] Iyengar, M. S., 1967. Protein from petroleum. Paper presented at Single-cell Protein Conference at MIT, Cambridge, Massachusetts.

BP protein designates the single-cell protein obtained from hydrocarbons by British Petroleum Company.

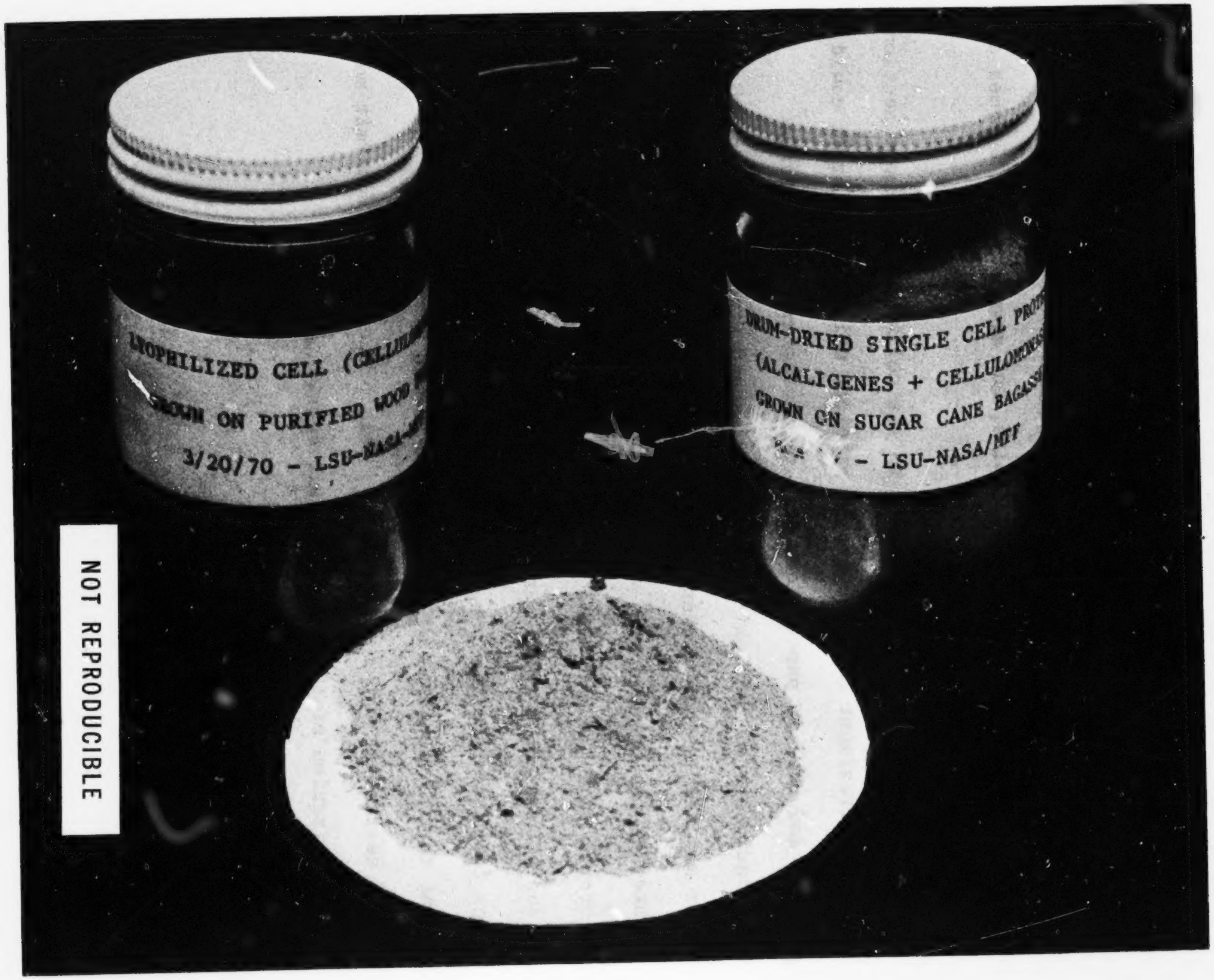
LYOPHILIZED CELL (CELLU
GROWN ON PURIFIED WOOD
3/20/70 - LSU-NASA
DRUM-DRIED SINGLE CELL PROT
(ALCALIGENES + CELLULOMONAS
GROWN ON SUGAR CANE BAGASS
- LSU-NASA/MTF
NOT REPRODUCIBLE

Figure 41. Single-cell protein, freeze-dried and drum-dried.

good. The product is easy to store, handle, and ship, and can easily be mixed with food or feed materials for nutritional uses.

Two by-product streams are currently produced by the pilot plant. The slurry underflow from the first mixer/settler, or the filter cake both contain the insoluble fiber fraction. In addition to the unused fiber, this solid stream also contains lignin, ash, salts, and some absorbed enzymes and organisms.

This unused fiber could be recycled to the cellulose treatment section for additional treatment and more nearly complete utilization, or it could be recovered and used in paper stock or the manufacture of chemicals.

The liquid stream that exits either the second mixer/settler or the centrifuge may either be recycled to the feed reslurry tank to replace makeup water or may be used as a nutrient solution for hydroponic gardening. A plant growth test has been made with this stream as a plant nutrient source, and it was found to be satisfactory.[42]

Owing to the relatively large volume of water used in the process, it is apparent that most of the effluent liquid should be recycled. In addition to decreasing water volume usage, this procedure would permit more efficient utilization of the inorganic salt nutrients.

ECONOMIC POTENTIAL

The importance or utility of any process depends upon its economics. It must be profitable to be commercially developed and industrially widespread. The future of SCP as a protein source for food or feed is involved not only in current supply and demand but also in the great protein needs of the future.

Many factors enter into the economics of microbial protein production. Some are typical chemical engineering economics that are familiar while others are not so familiar and require special consideration. Several factors can be defined that are probably the most important to the economics of microbial protein production, as follows.[43]

1. Raw material
    - a. Ease of collection to a central area
    - b. Availability to a given site
    - c. Bulk-handling properties
    - d. Seasonal fluctuations in availability
2. Sterility requirements
    - a. Microbial encroachment
3. Fermentation
    - a. Residence time in reactor (doubling time)
    - b. Cell concentrations attainable
    - c. Operating temperature (cooling water versus refrigeration)
    - d. Total oxygen requirements
    - e. Power requirements for mass transfer
    - f. Heats of reaction
    - g. Cell yields per lb of substrate consumed
    - h. Foaming tendency
4. Cell-harvesting techniques
    - a. High-speed centrifuges versus thickeners

5. Washing and purification techniques for removal of:
    a. Substrate residues
    b. Raw-material impurities
    c. Nucleic acids
    d. Metabolic by-products
6. Product value
    a. Percentage protein
    b. Limiting amino acid
    c. Digestibility

Factors such as drying costs and bagging and handling costs are omitted since these are generally typical and familiar to all chemical engineers. Certainly they will need to be included in the final analysis, but they must be about the same regardless of the type of product.

Many companies, both in the United States and abroad, are producing microbial proteins from hydrocarbons either in pilot plants or on a large scale. A good example is British Petroleum[44] with their pilot plant in Scotland, their 1-ton-a-day plant in India, or their new 30-million-ton-per-year plant in France. The Institut Francais des Petroles is already operating a 1-ton-per-day plant, while Esso Nestle's, Chinese Petroleum Corporation, and others are operating pilot units with obvious intentions of large plants in the future.

The cost of the carbon substrate used for SCP production varies not only with the substrate selected but also with geographical locations and the required purity of the starting material. For example, the cost of hydrocarbon substrate is doubled in going from gas oil at 1 cent per pound to purified n-paraffins at 2 cents per pound. The additional product purification costs of the proteins produced on gas oil may more than offset this initial 1-cent-per-pound difference in starting materials. As for geographical location, methane in the Gulf Coast area is about 0.25 cent per pound while the same material on the eastern seaboard would be about twice that.

The use of waste cellulose as a carbon substrate for microbial protein production does not present the same problems of purity and geographical location as those presented by hydrocarbon substrates. The production of SCP from high-purity cellulose is actually a deterrent to microbial growth. This same general conclusion was reached by Dr. N. J. King working with brown rot fungi grown on alkali-treated wood.[45] The waste cellulose requires, however, an alkali pretreatment for cellulose swelling and disruption of the lignin sheathing that protects the cellulose fibrils.

The geographical location of the plant would appear to have little bearing on the cost of the cellulose substrate since waste cellulose seems to be readily available everywhere. In rural areas the cellulose is available as corn cobs, rice hulls, wheat straw, sawdust, bagasse, and so on. In urban areas the solid wastes contain large portions of excelsior, books, newspapers, rags, towels, wood, and so forth. Not only are these wastes available, but also a credit rather than a debit may be given to the cellulose consumer for getting rid of unwanted solid wastes. One of the advantages of using bagasse as the source of cellulose is that it is collected at one central point, the sugar mill, and can be obtained for its fuel replacement value.

Bagasse costs have been calculated previously, and the raw material cost per pound of fermentable carbohydrate has been established at 1.5 cents.[6] The cost per ton of air-dry baled bagasse is about $18.90. This material contains about 10 percent moisture. These are conservative costs; they would probably be lower.

Productivity

Insufficient experimentation has been carried out to determine the maximum cell density that can be obtained with cellulose feed. The difficulty arises from the fact that two or more consecutive reactions must be carried on at about the same rate. If the enzymatic reaction goes too fast, then allosteric inhibition of feedback repression from the disaccharide causes a decrease in the cellulase activity. On the other hand, if rapid bacterial growth is reached too quickly (in a batch process), the log-phase growth rate becomes essentially irrecoverable. Doubling times of 2 to 4 hr have been observed with Cellulomonas, corresponding favorably to rates obtained on hydrocarbons. This means that holding time or dilution time would be about the same in both cases.

It has been shown that volumetric production efficiencies of about 0.16 g dry cell mass per liter of fermenter capacity per hour have been obtained with pure cultures, and as much as 0.51 has been obtained with symbiotic cultures (Table 13). Data have been collected and computed to compare these rates of SCP production with those of other comparable processes (Table 17). It is seen that these values range from less than 1/2 to 7 times the rate obtained in our plant. Limiting these values to those that have been experimentally verified, the range goes up to about five times our current rate. This was obtained with yeast growth and sulfite liquor. Comparable values for yeast grown on hydrocarbons should be in this same range.

The culture mass-doubling time of pure Cellulomonas cultures at about 3.5 to 3.7 hr is well within the range of values currently considered to be industrially feasible. The equilibrium cell density, however, is lower than in the yeast processes. That the equilibrium cell density can be improved from 0.5 g per liter is certain since the pilot plant has been operating at low substrate loadings; to what extent, however, is not known at this time.

A rather interesting discovery was made during previous research that could lead to improved fermentation economics although it is still too early

TABLE 17

COMPARABLE VOLUMETRIC PRODUCTION EFFICIENCIES FOR CONTINUOUS FERMENTATIONS

Organism	Substrate	VPE[a]	Reference
Yeast	Sulfite liquor	2.5 (Experimental)	21 (Inskeep)
Yeast	Acid-solubilized wood sugars	3.66 (Theoretical)	4 (Meller)
Yeast	Alkanes	3.46 (Theoretical)	44 (Wang)
Fungi	Cellulose	0.042 (Calculated from batch)	46 (Updegraf)
Mixed culture	Sulfite liquor	0.57 (Experimental)	47 (Amberg)
Bacteria (Cellulomonas)	Cellulose	0.162 (Experimental)	Table 13
Bacteria (Cellulomonas and Alcaligenes)	Cellulose	0.512 (Calculated from batch)	Table 13

[a] VPE = g of dry cell mass produced per liter of fermenter capacity per hr.

to be certain of all the aspects. During one fermentation of treated sugarcane bagasse, the rate and extent of visible cellulose breakdown increased markedly over the values from previous runs. The culture was found to be contaminated by an organism other than Cellulomonas. The second organism was isolated and identified by Drs. V. R. Srinivasan and Y. W. Han as of the genus Alcaligenes. A mixed culture of Cellulomonas and Alcaligenes was prepared and grown by them, and the culture was found to be composed primarily of Cellulomonas, and it exhibited better growth characteristics than either of the pure bacteria in the same media.[41] Concurrent experiments in the Chemical Engineering Department on measurement of the amount of solubilized carbohydrate present in the menstruum at any time showed that the amount solubilized remained essentially constant at 200 to 300 mg per liter in a pure culture of Cellulomonas. This knowledge, when combined with previous observations, indicates that what could be happening in the symbiotic culture was that the Alcaligenes was consuming the hydrolysis product that had previously been inhibiting enzyme activity. The inhibiting product is thought at the present time to be cellobiose. In other words, the enzyme activity that had previously been inhibited by the presence of the reaction product disaccharide in the menstruum was now free to proceed without inhibition.

The last fermentation run finished before the issuance of this report used Cellulomonas and Alcaligenes bacteria in a mixed-culture fermentation of treated bagasse. Cell densities increased five-fold over all previous pure culture runs, and growth rate was comparable. The calculated theoretical VPE value was almost five times higher than comparable values for pure cultures (Table 13). The only reason growth stopped at 6.24 g of cells per liter was lack of substrate. Higher cell densities should be obtainable.

In laboratory tests to seek other organisms that might be symbiotic with Cellulomonas, several types of cellobiose-metabolizing yeasts were grown with Cellulomonas in shake tubes. Most of the yeasts tested showed higher cell densities than either Cellulomonas or themselves grown separately in the same type media. None, however, showed as good growth as Cellulomonas and Alcaligenes (Table 18).

Amino acid patterns of Cellulomonas and Alcaligenes were determined for comparative purposes.[42] Alcaligenes showed a larger methionine content than Cellulomonas. Since methionine is the limiting amino acid in Cellulomonas protein metabolism, the addition of Alcaligenes should enhance the digestibility of the total SCP.

Work is continuing on the possibilities of symbiotic fermentation that could be of great benefit to both cell density and culture growth rates.

Cell Harvesting

The concentration of the cells in the effluent from the fermenter is very important when the cell concentration is to be increased in a desludging centrifuge. This is due to the fact that the cost of such equipment is normally based on the volumetric through-put rates. Furthermore, a great deal more power is required to handle the additional volume since the liquid must be subjected to forces 5,000 to 15,000 times gravity. This is much more noticeable in the harvesting of bacteria because their cell size is usually about one-fifth that of yeast, and power requirements are inversely proportional to the square of the particle size.

Initial tests on the separation of Cellulomonas have shown that these cells can be settled from suspension by adjusting the pH down to 5.2 or below or by adding a polyionic flocculent. A continuous thickener is used in the pilot plant in place of centrifuges. The power requirements and

TABLE 18

SYMBIOTIC GROWTH

Organism[a]	Growth[b] (Klett units)		
	0 hr	68 hr	92 hr
C	12	40	47
A	14	14	30
C + A	18	270	280
C + Yc1	22	60	78
C + Yc2	21	60	78
C + Yc3	16	60	70
C + Yc4	17	70	77
C + Yc5	17	62	55
C + Yc6	24	67	80
C + Yc7	17	52	65
C + Yc8	15	60	70
C + Yc9	20	80	85
C + Yc10	22	65	80
C + Yc11	17	100	110
C + Yc12	15	70	90
C + Yc13	15	100	110

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[a] C = Cellulomonas, A = Alcaligenes, Yc = cellobiose-utilizing yeast.

[b] Growth is the average of duplicate shake-tube culture.

maintenance on this equipment are extremely low compared with those for high-speed centrifuges. The cell cream from this vessel may then be concentrated much more economically by centrifugation.

Product Purification

The extent of purification required will depend upon the end use expected for the product. Probably the ultimate objective of essentially everyone's work in this area is to produce human food since this is a market that could afford to pay a good price for the product. The big stumbling block is nontoxicity and FDA approval. If this is the ultimate market for SCP grown on gas-oil with its attendant conglomeration of products that cannot be consumed by bacteria, then FDA would be quick to recognize that such products would not be very nourishing to human beings. This means, then, that for these products a large cost will be involved in solvent extraction or whatever to remove these components from the final product.

Single-cell protein grown on hydrocarbon then will have some rather costly purification steps unless an organism can be found that will easily disengage itself from the residual hydrocarbons. The FDA has gone on record in the past as ruling that the addition of as little as 200 ppm of mineral oil to the human diet is objectionable.

Microbial proteins grown on cellulose may not encounter such severe restrictions as products grown on other substrates, because cellulose is not considered objectionable in the human diet. In fact, it is often added to the diet as a bulking agent in the form of a water-soluble derivative. Some of the undigested lignin remaining in the effluent stream is certainly going to go out with the product, but lignin also is not particularly objectionable in the human diet, since one already gets fair quantities of it in certain garden vegetables and fruits. The lignin passes through the digestive tract without being assimilated.

Perhaps the largest worry that all SCP producers have is the presence of very large amounts of nucleic acid in the protein product. Nucleic acids have a deleterious effect on rats, causing gastric disturbances, skin rash, and other effects, and very little is known about the tolerance limits of such products in the human diet.

Product Value

The market value of the product will determine how high the production costs can go. It would appear at the moment that very few single-cell proteins will be able to compete with soybean protein *per se*. Still, there are many factors that contribute to the value of protein other than adding up the total nitrogen content and multiplying by 6.25. It turns out that in the human diet the value of a protein is based on the digestibility of the product, and this is usually limited by either the difficulty of cell wall rupture or the amount of the limiting amino acid, or both.

The quality of a given protein is based on how much weight gain results per gram of protein consumed. This is variously determined as PER (protein efficiency ratio), or the amount of weight gain per gram of protein intake; as BV (biological value); as NPU (net protein utilization); and so forth. In the final analysis, the data that would be most desirable would be the protein quality divided by the cost or the weight gain per unit of cost for each of the various proteins. Unfortunately, these data appear to be rather limited for unconventional proteins.

Summary of Product Cost

It has been impossible to obtain a detailed cost analysis on the production of SCP on hydrocarbon substrate from one of the large producers because of the competitive nature of this endeavor. About the only cost information available appears to be the final selling price, and even that has to be looked at with some skepticism because it could change with supply and demand. Cost data available show quite a wide range (Table 19). Esso Research and Engineering has indicated an approximate selling price of 17 cents per pound of 50 percent protein single cells that have been grown on n-paraffin. British Petroleum has indicated a price in the range of 10 to 20 cents per pound for 50 percent protein grown on gas-oil. Soybean proteins are quoted at a price of 6 to 7 cents a pound on the same basis while fish flour is in the range of 15 to 20 cents per pound. It should be obvious from these figures that if the market for SCP is to be animal feed supplement, then the price to beat is the 6 to 7 cents per pound for soybean flour.

The current operating data for the pilot plant do not permit an extensive economic analysis. A key point, however, in the evaluation of any SCP process is the VPE or fermenter productivity value. Most economic analyses in the literature give a VPE of from 1.5 to 3.5 as an economic break-even point. Our current VPE of from 0.1 to 0.5 is considerably below this point. The key to enhancement of the processing economics is an increase in the VPE value. Our current VPE would make it necessary to operate large fermenters and cell recovery equipment, and this hurts the present economic picture.

Since, however, the little optimization done so far has yielded favorable results in production rates and efficiencies, it is felt that VPE values for this process can be raised to a competitive range. Moreover, symbiotic-culture growth has shown great potential, and other economic savings will be realized with further work.

TABLE 19

COST OF CONVENTIONAL AND UNCONVENTIONAL PROTEIN[a]

Food product	Price of product (¢/lb)	Percent protein	Price of protein (¢/lb)
Conventional protein foods:			
Wheat flour	5	12	42
Skim milk powder	15	36	43
Fish, dried	14	37	39
Cheese	32	24	133
Chicken	26	15	173
Beef	21	12	175
Eggs	24	11	211
Less conventional protein foods:			
Cottonseed flour	7	55	12
Soy protein flour	7	52	13
Fish protein concentrate	12.5	85	14
Peanut Cake	7	42	17

TABLE 19 (Continued)

COST OF CCNVENTIONAL AND UNCONVENTIONAL PROTEIN[a]

Food product	Price of product (¢/1b)	Percent protein	Price of protein (¢1/b)
Unconventional protein foods:			
Algae	3	50	6
Yeast (petroleum)[b]	6-8	50	12-16
Leaf protein	37-47	50	74-94
Spirulina	18	65	28
Yeast (vegatable)	10-14	50	20-28
Bacteria (cellulose)[c]	10-15	50-55	20-30

[a] All prices, except where noted, are from Abbott, J. C., 1966. Unconventional protein. Presented at engineering research conference at Santa Barbara, California.

[b] Wang, D. I. C. Proteins from petroleum. Chemical Engineering, p. 99, Aug. 26, 1968.

[c] Data from this report, based on current operating data.

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